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TRANSMITTAL FORM

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First Named Inventor	Jan C. SIMON
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Examiner Name	Ruth A. DAVIS
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ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 (CFR) 1.52 or 1.53
- ☐ Drawing(s)
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- ☐ After Allowance communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☒ Appeal Communication to Group **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s), please identify below):

Response to Notice of Non-Compliance submitting amended brief

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

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Signature	Patricia D. Granados
Date	March 2, 2005

CERTIFICATE OF TRANSMISSION/MAILING

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No: 24741-1525

In re patent application of:
In re Jan C. SIMON *et al.*

Confirmation No.: 2389

Application No.: 09/856,694

Art Unit: 1651

Filed: August 13, 2001

Examiner: Ruth A. DAVIS

For: HYPERFORIN AS CYTOSTATIC AGENT AND HYPERFORIN OINTMENT OR CREAMS AS

RESPONSE TO NOTICE OF NON-COMPLIANCE WITH THE REQUIREMENTS OF 37 CFR 41.37(d)

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicants herein respond to the Notice of Non-compliance with the Requirements of 37 CFR 41.37(d) mailed from the U.S. Patent and Trademark Office January 31, 2005, a response to which is due March 2, 2005. Applicants believe that no fees are due at this time. If any fees are due, Applicants herewith authorize the Commissioner to charge the undersigned's Account No. 08-1641.

Applicants submit herewith a substitute Appeal Brief that has been amended in compliance with the requirements of 37 CFR 41.37(d).

Respectfully submitted,

Date March 2, 2005

By Patricia D. Granados
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE HONORABLE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Jan C. SIMON *et al.*

Serial No.: 09/856,694

Filed: August 13, 2001

Art Unit: 1651

Attorney Docket No.: 24741-1525

For: HYPERFORIN AS CYTOSTATIC AGENT AND HYPERFORIN OINTMENT OR CREAMS AS APPLICATION FORM

SUBSTITUTE BRIEF ON APPEAL

Appeal from the Primary Examiner

Heller Ehrman White & McAuliffe LLP
1717 Rhode Island Avenue, NW
Washington, DC 20036-3001

BRIEF ON APPEAL

Appellants appeal the August 6, 2004 final rejection (the "Final Rejection") of the captioned application to the Board of Patent Appeals and Interferences. Appellants filed a Notice of Appeal on October 20, 2004.

I. REAL PARTY IN INTEREST

UNIVERSITAETSKLINIKUM FREIBURG, as assignee, owns the entire right, title and interest in the captioned application and, therefore, is the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

Appellants are aware of no other current appeals or interferences pertaining to the instant invention. Appellants previously filed a Brief on Appeal for this application, but the Patent and Trademark Office reopened the application for prosecution so as to address a defect with one of its references.

III. STATUS OF CLAIMS

Claims 36-45 and 56 are on appeal. These claims stand finally rejected, as indicated in the final rejection. A copy of the claims on appeal is attached.

Claims 1-19 were canceled in a Preliminary Amendment on May 24, 2001. Claims 20-35 were canceled in an Amendment and Response under 37 CFR §1.111 on February 21, 2002. Claim 55 was canceled in an Amendment under 37 CFR §1.116 on August 2, 2002. Claims 46-55 were canceled, and claims 36, 38 and 56 were amended in an Amendment under 37 CFR §1.111 on May 26, 2004.

IV. STATUS OF AMENDMENTS

An amendment was filed October 20, 2004 after the final rejection mailed August 6, 2004. This amendment was not entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The invention relates to a method for treating a condition, comprising administering to a subject in need thereof an effective amount of a composition consisting of (a) pharmaceutically acceptable carrier and (b) active agent consisting of (i) hyperforin or (ii) hyperforin and hypericin, wherein said condition is an inflammatory skin condition, a precancerous condition, a geriatric skin condition, or a microbial skin infection. (Specification at 7, line 15; at 9, line 31 to 10, line 12)

In one embodiment, the condition is eczema; in another, the condition is exsiccation eczemas, hyperkeratotic hand and foot eczemas, contact eczemas, atopic dermatitis, neurodermatitis, lichen simplex, prurigo simplex, lymphomas, leukemia, an epithelial pre-cancerous condition, tumor metastases, or an epithelial tumor. (Specification at page 10, lines 5-11)

The subject of the treatment may be a mammal. (Specification at page 10, lines 18-21)

The method of the invention may use a composition in the form of a topical ointment, and the effective amount is at least 15 μg hyperforin per ml of the composition (Specification at 7, lines 4-8). In another embodiment, the effective amount of such a composition is 0.02-20 mg hyperforin per ml of the composition (Specification at page 7, lines 4-11); in another, the effective amount is 1-20 mg hyperforin per ml of the composition (Specification at page 7, line 13); in another, the effective amount is either at least 10 mg (Specification at page 7, line 13) or at least 15 μg hypericin per ml of the composition (Specification at page 7, line 20). In the method of the invention, the hyperforin may be at least 90% pure (Specification at page 12, lines 17-18).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issues in this appeal are:

- Whether claims 36-45 and 56 would have been obvious over The Hypericum Homepage (*Hypericum & Depression,* Bloomfield *et al.*, Copyright 1996, Prelude Press, Editor J. Sedillos) in view of *The Merck Manual* (1995-2002) within the meaning of 35 U.S.C. § 103(a).

- Whether claims 36, 38-45, and 56 would have been obvious over Valavichyus, "Antitumor Activity of Medicinal Plants from the Lithuanian SSR, USSR 6, Common St. John's Wort Chamomilla Recutita" (*Abstract from BIOSIS*, 1986) within the meaning of 35 U.S.C. § 103(a).

- Whether claims 36, 38-45, and 56 would have been obvious over Valavichyus in view of HHP and/or DeCosterd, *Helvetica Chimica Acta*, 72:464-471 (1989) within the meaning of 35 U.S.C. § 103(a).

The rejected claims do not stand or fall together. Claim 36 is independent and recites a method of treating a condition selected from a group of conditions consisting of inflammatory skin conditions, a precancerous condition, a geriatric skin condition and a microbial skin infection. Claim 38 depends from claim 36 and recites, among other things, leukemia and lymphoma. Although Appellants attempted to make claim 38 independent, and also have a separate claim directed to leukemia and lymphoma, the Examiner did not enter the amendment. Appellants believe that at least two embodiments in claim 38, *i.e.* leukemia and lymphoma, are separately patentable. Although Appellants acknowledge that they could have filed a Request for Continued Examination instead of filing this Brief on Appeal, Appellants believed that this case is otherwise ready for appeal and would welcome a remand to correct the claim dependency issue.

Appellants also believe that the invention of claim 56 is separately patentable; none of the art of record suggests the purity level recited in this claim.

VII. ARGUMENT

A. Claims 36-45 and 56 would not have been rendered obvious by The Hypericum Homepage in view of The Merck Manual within the meaning of 35 U.S.C. § 103(a).

1. The Rejected Claims

Independent claim 36 recites a method for treating a condition, comprising administering to a subject in need thereof an effective amount of a composition consisting of (a) pharmaceutically acceptable carrier and (b) an active agent consisting of (i) hyperforin or (ii) hyperforin and hypericin, wherein said condition is selected from the group consisting of an inflammatory skin condition, a precancerous condition, a geriatric skin condition, and a microbial skin infection.

Dependent claims 37-38 specify administration of the compound for treating specific conditions. Dependent claim 39 specifies the subject as a mammal. Dependent claims 40-45 prescribe that the composition be a topical ointment and that the effective amount be at least 15 micrograms of hyperforin/ml, 0.02-20 mg/ml or 1-20 mg/ml; or 15 micrograms/ml or 20-150 micrograms/ml hypericin. Claim 56 specifies that the hyperforin be at least 90% pure.

2. The PTO's Case

The U.S. Patent and Trademark Office (the "PTO") asserts that the Hypericum Home Page ("HHP") teaches that extracts of St. John's Wort, which contains hyperforin and hypericin, exhibits anti-inflammatory and antibacterial effects when applied externally or topically, and specifically teaches that hyperforin is attributed with anti-inflammatory and antibacterial effects (Final Rejection at 3). The PTO argues that although HHP does not teach a method for treating an inflammatory condition with the claimed effective amounts or for the specific conditions, it would have been obvious to one of ordinary skill in the art to use hyperforin and/or hyperforin and hypericin to treat inflammatory conditions because of the disclosed anti-inflammatory effects (Final Rejection at pages 3-4).

The PTO further argues that it would have been obvious to one of ordinary skill in the art to optimize effective volumes and concentrations as a matter of routine experimentation (Final Rejection at page 4), and that it would have been obvious to one of ordinary skill in the art to include a pharmaceutical carrier.

Finally, the PTO asserts that one of ordinary skill in the art would have been motivated to use hyperforin in a method for treating inflammatory conditions with a reasonable expectation of success because of hyperforin's known benefits, as disclosed by HHP (Final Rejection at page 4). The PTO cites the *Merck Manual* ("Merck"), Shroot *et al.* (U.S. Patent No. 5,151,534), and Lacefield *et al.* (U.S. Patent No. 4,021,553) as evidence that one of ordinary skill in the art would have known that eczemas are inflammatory diseases (Final Rejection at page 4). According to the PTO, one of ordinary skill in the art would have been motivated to combine HHP and Merck, and utilize hyperforin in a method for treating inflammation and eczemas with a reasonable expectation of success.

3. Appellants' Response

The PTO has not presented a *prima facie* case of obviousness. Under the relevant law, the standard for assessing obviousness is (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device or carry out the claimed process and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *In re Vaeck*, 947 F. 2d 488, 493, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). The PTO has not met this burden.

St. John's Wort extract is not the same thing as hyperforin or hyperforin with hypericin. HHP discusses St. John's Wort extract. Specifically, it states that St. John's Wort contains at least ten different components. It describes some of the therapeutic uses of St. John's Wort, including the use for treating depression. It notes that it has been reported that externally applied St. John's Wort has anti-inflammatory and

antibacterial effects and that such effect has been attributed to the hyperforin in the St. John's Wort extract.

It is clear from the HHP disclosure that St. John's Wort extract is a complicated combination of many different ingredients. This is supported by another reference cited by the PTO in connection with the rejection discussed below, Chavez *et al.*, which states that "[t]he chemical constituents of St. John's Wort are complex, numerous and diverse" and that "[t]he amount of the constituents is related to the harvesting period, the drying process and the storage." (Chavez at 1622). Although HHP mentions hyperforin as possibly being responsible for St. John's Wort having an anti-inflammatory property when used externally, it does not teach the use of a purified, effective amount of hyperforin in a pharmaceutical composition for use in treating an inflammatory skin condition, a precancerous condition, a geriatric skin condition or a microbial skin infection. The PTO admits these deficiencies in HHP. It relies upon what would be "obvious to the skilled artisan" to optimize the effective amount and put it into a carrier" to complete its case.

Appellants also note that the PTO acknowledges that the art considers St. John's Wort oil to be hypercin-free (Final Rejection at page 8, first paragraph). Thus, the PTO must acknowledge that the embodiment whereby hyperforin and hypercin are present, is not addressed by the art of record.

Appellants also point out that it is not clear from HHP what type of inflammation was treated with St. John's Wort, whether it was inflammation due to a skin condition or whether there was any evidence that hyperforin was actually responsible for the anti-inflammatory response. After all, according to HHP, St. John's Wort contains at least nine other ingredients. The PTO's primary reference is silent on these points.

In fact, the PTO has asserted that "...at the time of the claimed invention, it would have been obvious to one of ordinary skill in art to optimize effective volumes and concentration as a matter of routine experimentation" and that one "would have been

motivated to use hyperforin in a method of treating external anti-inflammatory conditions with a reasonable expectation of success because of its known benefit as disclosed by HHP." (Final Rejection at page 4, first paragraph, at pages 6-7, bridging paragraph). However, a showing of motivation requires more than a blanket assertion of motivation without anything more. The Federal Circuit has emphasized this need in *In re San-su Lee*, 277 F. 3d 1338, 61 USPQ2d 1430 (Fed. Cir. 2002). The court stated that "...the factual showing for motivation is material to patentability, and could not be resolved on subjective belief and unknown authority." *Id.*

Additionally, the PTO's reliance upon Merck and the other secondary references does not cure the limitations in the HHP reference. These secondary references disclose various types of inflammatory skin disorders, *e.g.*, eczema, lichen simplex, chronic dermatitis. The PTO asks us to assume that a treatment of inflammation of one type would be a treatment for an inflammation of another type. This simply isn't true. Even if the HHP reference disclosed the use of hyperforin in a pharmaceutical to treat inflammation from a skin disorder (which it does not), there is no scientific reason to believe such treatment would be suitable for the specific disorders listed in claim 36 and in the rejected dependent claims. The PTO has failed to support the assumption that all substances that have anti-inflammatory properties are effective and safe for treating specific diseases that produce an anti-inflammatory response. Thus, one of ordinary skill in the art would not have an expectation of success based upon the PTO's selected combination of teachings.

Finally, Appellants argue that none of the cited references teach or suggest the use of hyperforin or hyperforin and hypericum for treating lymphoma or leukemia (claim 38), nor has the PTO made such an argument. The PTO's rejection is silent with regard to these embodiments. As such, the rejection lacks support with regard to this embodiment. As noted above, Appellants attempted to put these embodiments into an independent claim and to also have a separate claim directed to leukemia and lymphoma. However, the PTO refused to enter such amendments.

B. Claims 36, 38-45 and 56 would not have been rendered obvious over Valavichyus within the meaning of 35 U.S.C. § 103(a).

1. The Rejected claims

Claims 36, 38-45 and 56 have been discussed above.

2. The PTO's Case

The PTO asserts that Valavichyus teaches that extracts of St. John's Wort, specifically oil extracts, inhibit growth of sarcoma cells and tumor growth in animals. The PTO further asserts that it was well known in the art that oil preparations of St. John's Wort are hypericin-free and contain high concentrations of hyperforin, citing Chavez, *Monographs on Alternative Therapies in Hospital Pharmacy* 32: (12): 1621-1632 (1997), and that plant oils were used as pharmaceutical carriers (Final Rejection at pages 7-8).

The PTO concludes that although Valavichyus does not teach the method, volume, concentrations, mode of administration, or purity of hyperforin, one of ordinary skill in the art could determine these amounts by routine experimentation. According to the PTO, one of ordinary skill in the art would have been motivated by routine practice to optimize the effective amounts of Valavichyus with a reasonable expectation for successfully treating cancer.

3. Appellants' Response

The PTO's obviousness rejection over Valavichyus is defective as a matter of fact and law. The PTO's error in fact relates to its interpretation of the cited art and its failure to acknowledge that claim 36 has been amended to remove "cancer". In any event, the cited references do not disclose what the PTO claims they do. Accordingly, one could not arrive at the invention from reading Valavichyus alone or in combination with Chavez, and the rejection is therefore unsupportable as a matter of law.

Specifically, the entire Valavichyus abstract states:

> The effect of oil extracts of the St. John's wort-hypericum perforatum and Chamomilla recutita on the growth of sarcoma 45 and Cholangioma PC-1 was studied in rats. The administration of the extracts inhibited the growth of tumors and increased the body weight of the animals. Data were presented on the effect of various doses of the extracts on the inhibition rate of the tumors.

It is not clear from this disclosure whether an oil extract of St. John's Wort or a combination of such extract with an oil extract of Chamomilla recutita was tested on mice tumors. Further, it is not clear what was in the oil extract of St. John's Wort. Was it only hyperforin, or was it a combination of ingredients? How was the oil extract prepared? Was it prepared using olive oil on flowers, as described in Chavez, or was it prepared some other way? Appellants have shown through the references of record in this case that St. John's Wort is a complicated plant containing many different ingredients. Different extracts from different parts of the plant contain different components and these components change with time and storage. The PTO attempts to address this issue by relying upon the teachings of Chavez.

Specifically, at page 1622, Chavez teaches that "typically" oil preparations of St. John's Wort are prepared by extracting the flower with olive oil. It further states that such oil preparations are hypericin free and contain lipophilic compounds, including "sufficiently high" concentrations of hyperforin. What Chavez does not teach, however, is what else is the oil extract. What are the other lipophilic compounds? Also, it is not clear what is meant by "sufficiently high concentrations" of hyperforin. For what is it sufficiently high?

It is clear that neither of the cited references teaches nor suggests, either alone or in combination, a pharmaceutical composition comprising hyperforin that is 90%

pure, as recited in claim 56. The PTO reads information into the cited references that is not actually there and then combines the alleged teachings to arrive at the invention. As such, the PTO's argument is based upon hindsight knowledge of the invention, which is an impermissible basis for an obviousness rejection.

As noted above, Appellants have amended claim 36 to remove "cancers." Thus, this rejection is arguably inapplicable with regard to claim 36. Also, claim 36 is directed to a method of treating by administering an effective amount of a composition **consisting of** hyperforin or **consisting of** hyperforin **and** hypercin and a pharmaceutically acceptable carrier. The cited references do not teach or suggest one or both of these active agents and the exclusion of other active agents. One could not arrive at the claimed invention by combining Valavichyus with Chavez. Nothing in either reference directs the skilled artisan to the use of hyperforin or hyperforin and hypercin in a pharmaceutical composition to treat any type of cancer, particularly those recited in the claims on appeal. Nothing in either reference suggests what might be an effective amount of such compositions. Although Valavichyus might invite experimentation in the field of the invention, such an invitation cannot be a basis for an obviousness rejection.

The PTO's obviousness case is defective for yet another reason. Claims 36 and the other claims dependent thereon recite "a pharmaceutically acceptable carrier." This claim element is not taught or suggested by the cited references, and in view of the specification, it is improper to construe Appellant's claims to equate St. John's Wort oil or extract with "a pharmaceutically acceptable carrier."

Even if one assumes that Valavichyus suggests oil extracts generally, such extracts, without more information, are not pharmaceutically effective. The science presented in the specification shows that oil extracts of St. John's Wort are undesirable. The specification shows that actual skin cells (not a culture of cells many generations removed from the reality of disease processes in humans) were studied directly. That is, when Appellants treated real skin of living humans, and then studied cell samples scraped from those subjects, the plant oil (St. John's Wort oil) failed miserably and

clearly was shown to be a bad carrier (Specification at page 19, last paragraph through the middle of page 20)

Appellants have obtained data from real *in vivo* studies that shows that Valavichyus's conclusions are wrong. A skilled artisan following Valavichyus would be led in the wrong direction. To the extent that Valavichyus is relevant, it teaches away from the claimed invention. Such evidence of leading away is a further indication of unobviousness.

Appellants reiterate that the specification provides ample information regarding the desirable aspects of pharmaceutically effective carriers. As described in the specification (see Example 11 and associated text), St. John's Wort oil is not a pharmaceutically effective carrier. In the context of Appellants' specification, which teaches how to use the claimed invention, there is no reason to think that plant oils *per se* somehow are pharmaceutically acceptable carriers. On the contrary, the oil studied (St. John's Wort) was not acceptable and it was found that the active ingredients can be combined with ethanol and cream, as described on page 6, first paragraph of the specification, ethanol and greasy ointment base (second paragraph of page 6 of the specification). Ethanol is particularly useful for the pharmaceutically effective carrier (page 8, second paragraph of the specification), and "plant extracts" such as plant oils, if used, are used as ingredients, not carriers *per se*, as mentioned on page 8 lines 19-22. Crude plant oil extracts generally are not pharmaceutically acceptable. The specification at pages 8 and 9 describes carriers that are acceptable. Plant oil extracts are not in this list.

The PTO's assumption that "[i]t was also known in the art that plant oils were used as pharmaceutical carriers" (Final Rejection page 5) is simply not correct. A pharmaceutically acceptable carrier is not a plant oil extract. In fact, as discussed above, the specification provides data showing that a plant oil extract studied was not acceptable and that the plant oil has to be blended with acceptable materials (Specification at page 9, first three full paragraphs and Example 11).

Appellants further maintain that the above-discussed data in the specification bolster the non-obviousness of their invention. Even if the PTO had presented a *prima facie* case of obviousness, the evidence presented in the specification would rebut such case. The specification teaches "pharmaceutically acceptable" carriers such as "ointment or cream" as, for example, stated on page 10, line 37. Particular advantages of this acceptable carrier are also stated on the bottom of page 37. The effects of the ointment and creams (representative pharmaceutically acceptable carriers) "is superior to that of the known St. John's Wort oil" as stated on page 11, lines 9 to 10 of the specification. This effect was previously generally unknown and unexpected. Another effect is that "penetration of active compounds" from these particular pharmaceutically acceptable carriers "is superior to that of active compounds from oils." Applicants note in this context that the word "oils" includes plant oils such as plant oil extracts. Such plant oil preparations are NOT included within the group of pharmaceutically acceptable carriers, as is stated above.

Here, Appellants discovered to their surprise through investigation that the St. John's Wort ointment (*i.e.*, with a pharmaceutically effective carrier) "brings about an inhibition of proliferation (of epidermal cells). On the other hand, the use of St. John's Wort oil results in an increase in proliferation" as seen in the data of Figure 5 from the specification (see page 20, lines 14-18). Clearly, the claimed compositions (not with the plant oil as carrier but with an acceptable carrier) exhibited highly beneficial activity as unexpected results in comparison with the "natural" product promoted and taught by Valavichyus (and HHP). Although these unexpected results are strong evidence of unobviousness, in the Final Action at page 9, first paragraph, the PTO states that Appellants failed to present "evidence" of unexpected results. Appellants counter that the PTO's failure to acknowledge and give weight to the evidence in the specification is improper and provides yet another basis to appeal this rejection.

C. Claims 36, 38-45 and 56 would not have been obvious over Valavichyus in view of HHP and/or DeCosterd within the meaning of 35 U.S.C. § 103(a).

1. The Rejected Claims

Claims 36, 38-45, and 56 have been discussed above.

2. The PTO's Case

The PTO rejects all the claims as being obvious over Valavichyus in view of HHP or DeCosterd, the secondary references being cited for allegedly teaching that extracts of St. John's Wort have anti-tumor properties. Specifically, the PTO states that "DeCosterd teaches extracts of Hypericum inhibit growth of colon carcinomas" and further teaches derivatives of hyperforin that exhibit the growth-inhibiting activity (Final Rejection at page 10). From this the PTO concludes that at the time of the invention, hyperforin, derivatives thereof, and extracts of Hypericum were well known as effective agents against cancer of various kinds.

3. Appellants' Response

Appellants again point out that the claims on appeal do not recite "cancer." Thus, the applicability of this rejection is questionable. However, aside from this point, nothing in DeCosterd cures the deficiencies in the PTO's case, as set forth above in connection with the discussion of HHP and Valavichyus. DeCosterd teaches the isolation of two new compounds, hyperevolutin A and hyperevolutin B from the root bark of Hypericum revolutin VAHL. These compounds showed growth inhibitory activity against *in vitro* colon carcinoma cell line. Such a report does not direct the skilled artisan to Appellants' invention. Rather, it invites experimentation and further investigation. As such, it does not support an obviousness rejection.

D. CONCLUSION

It is respectfully requested that the Board pass the presently rejected claims on to allowance.

Respectfully submitted,

Date March 2, 2005

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VIII. CLAIMS APPENDIX

Claims 36-45 and 56 are currently on appeal.

36. A method for treating a condition, comprising administering to a subject in need thereof an effective amount of a composition consisting of (a) pharmaceutically acceptable carrier and (b) active agent consisting of (i) hyperforin or (ii) hyperforin and hypericin, wherein said condition is selected from the group consisting of an inflammatory skin condition, a precancerous condition, a geriatric skin condition, and a microbial skin infection.

37. The method according to claim 36, wherein the condition is eczema.

38. The method according to claim 36, wherein said condition is selected from the group consisting of exsiccation eczemas, hyperkeratotic hand and foot eczemas, contact eczemas, atopic dermatitis, neurodermatitis, lichen simplex, prurigo simplex, lymphomas, leukemia, an epithelial pre-cancerous condition, tumor metastases, and epithelial tumor.

39. The method according to claim 36, wherein said subject is a mammal.

40. The method according to claim 36, wherein said composition is in the form of a topical ointment and said effective amount consists of at least 15 μg hyperforin per ml of the composition.

41. The method according to claim 36, wherein said composition is in the form of a topical ointment and said effective amount is 0.02-20 mg hyperforin per ml of the composition.

42. The method according to claim 41 wherein said effective amount is 1-20 mg hyperforin per ml of the composition.

43. The method according to claim 42 wherein said effective amount is 10 mg hyperforin per ml of the composition.

44 The method according to claim 36, wherein said effective amount is at least 15 μg hypericin per ml of the composition.

45. The method according to claim 36, wherein said effective amount of hypericin is 20-150 μg hypericin per ml of the composition.

56. The method of claim 36, wherein said hyperforin is at least 90% pure.

IX. EVIDENCE APPENDIX

The following is a list of references entered by the Examiner and/or relied upon by Appellant in this appeal, along with a statement setting forth where in the record that evidence was entered by the Examiner and/or the Appellant. Copies of each piece of evidence are provided herewith.

Reference	Location in the Record
1. Angeli (WO 91/15218).	Non-Final Office Action of 10/22/01 (pages 4-5); Amendment Under 37 CFR §1.111 filed 2/21/02 (pages 6-7).
2. Chavez, *Monographs on Alternative Therapies in Hospital Pharmacy* 32(12), 1621 (1997).	Final Office Action of 5/3/02 (pages 9-10; Non-Final Office Action of 11/5/02 (pages 9, 11); Final Office Action of 6/3/03 (page 5); Final Office Action of 6/3/03 (page 7); Appeal Brief filed 11/3/03 (pages 6-7, 9-11); Non-Final Office Action of 2/26/04 (pages 8-9, 11); Amendment Under 37 CFR § 1.111 filed 5/26/04 (pages 5-6); Final Office Action of 8/6/04 (pages 8-10); Appeal Brief filed 10/20/04 (pages 5 and 7); Appeal Brief filed 12/20/04 (pages 7 and 9-11).
3. DeCosterd, *Helvetica Chimica Acta*, 72: 464-471 (1989).	Final Office Action of 5/3/02 (pages 10-11); Amendment Under 37 CFR § 1.116 filed 8/2/02 (pages 8-9); Non-Final Office Action of 11/5/02 (pages 10-12); Amendment Under 37 CFR § 1.111 filed 3/5/03 (page 6); Final Office Action of

Reference	Location in the Record
	6/3/03 (pages 6, 8); Appeal Brief filed 11/3/03 (pages 3, 14); Non-Final Office Action of 2/26/04 (pages 10, 12); Amendment Under 37 CFR § 1.111 filed 5/26/04 (pages 8-9); Final Office Action of 8/6/04 (pages 9-11); Appeal Brief filed 10/20/04 (page 8); Appeal Brief filed 12/20/04 (pages 4 and 15).
4. Khan *et al.* (GB patent No. 2311009 A)	Non-Final Office Action of 10/22/01 (pages 5-6, 8-9); Amendment Under 37 CFR §1.111 filed 2/21/02 (pages 6-7).
5. Khwaja *et al.* (WO 97/39355).	Non-Final Office Action of 10/22/01 (pages 8-9); Amendment filed 2/21/02 (pages 7-8).
6. Lacefield *et al.* (U.S. Patent No. 4,021,553).	Non-Final Office Action of 11/5/02 (page 4); Final Office Action of 6/3/03 (page 4); Appeal Brief filed 11/3/03 (page 5); Non-Final Office Action of 2/26/04 (page 4); Amendment filed 5/26/04 (page 4); Final Office Action of 8/6/04 (page 4); Appeal Brief filed 10/20/04 (page 5); Appeal Brief filed 12/20/04 (page 6).
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Reference	Location in the Record
8. Shroot *et al.* (U.S. Patent No. 5,151,534).	Non-Final Office Action of 11/5/02 (page 4); Final Office Action of 6/3/03 (page 4); Appeal Brief filed 11/3/03 (page 5); Non-Final Office Action of 2/26/04 (page 4); Amendment filed 5/26/04 (page 4); Final Office Action of 8/6/04 (page 4); Appeal Brief filed 10/20/04 (page 5); Appeal Brief filed 12/20/04 (page 6).
9. Valavichyus "Antitumor Activity of Medicinal Plants from the Lithuanian SSR, USSR 6, Common St. John's Wort Chamomilla Recutita" (*Abstract from BIOSIS*, 1986).	Non-Final Office Action of 5/22/01 (pages 8-9); Amendment Under 37 CFR § 1.111 filed 2/21/02 (pages 7-8); Final Office Action of 5/3/02 (pages 3-4, 8-11); Amendment Under 37 CFR § 1.116 filed 8/2/02 (pages 4, 8-9); Non-Final Office Action of 11/5/02 (pages 8-13); Amendment Under 37 CFR § 1.111 filed 3/5/03 (page 6); Final Office Action of 6/3/03 (pages 5-8); Appeal Brief filed 11/3/03 (pages 3, 8-9, 11, 13-14); Non-Final Office Action of 2/26/04 (pages 2, 7-12); Amendment filed 5/26/04 (pages 6-9); Final Office Action of 8/6/04 (pages 7-10); Appeal Brief filed 10/20/04 (pages 6-8); Appeal Brief filed 12/20/04 (pages 4, 10-15).
10. The Hypericum Homepage (*Hypericum & Depression,* Bloomfield *et al.*, Copyright 1996, Prelude Press, Editor J. Sedillos),	Non-Final Office Action of 5/22/01 (pages 8-9); Amendment Under 37 CFR § 1.111

Reference	Location in the Record
http://www.hypericum.com/hyp20.htm) (HHP).	filed 2/21/02 (pages 7-8); Final Office Action of 5/3/02 (pages 5-8, 11); Amendment Under 37 CFR § 1.116 filed 8/2/02 (pages 5-9); Non-Final Office Action of 11/5/02 (pages 3-5, 10-12); Amendment Under 37 CFR § 1.111 filed 3/5/03 (pages 3-5); Final Office Action of 6/3/03 (pages 3-4; 6-9); Appeal Brief filed 11/3/03 (pages 3, 5-7, 13-14); Non-Final Office Action of 2/26/04 (pages 3-6, 10-12); Amendment Under 37 CFR § 1.111 filed 5/26/04 (pages 5-6, 8-9); Final Office Action of 8/6/04 (pages 3-6, 9-11); Appeal Brief filed 10/2/04 (pages 4, 6, 8); Appeal Brief of 12/20/04 (pages 4, 6-8, 14-15).
11. *The Merck Manual* (1995-2002). (http://www.merck.com/pubs/mmanual/section10/chapter111/111a.htm, printed April 29, 2002).	Final Office Action of 5/3/02 (page 6, 8); Amendment Under 37 CFR § 1.116 filed 8/2/02 (page 7); Non-Final Office Action of 11/5/02 (pages 3-5, 7); Amendment Under 37 CFR § 1.111 filed 3/5/03 (page 3); Final Office Action of 6/3/03 (pages 3-4, 9); Appeal Brief filed 11/3/03 (pages 3, 5-7); Non-Final Office Action of 2/26/04 (pages 3-5, 7); Amendment Under 37 CFR § 1.111 filed 5/26/04 (pages 4-6); Final Office Action of 8/6/04 (pages 3-4); Appeal Brief filed 10/20/04 (page 5);

Reference	Location in the Record
	Appeal Brief filed 12/20/04 (pages 6, 8).

X. RELATED PROCEEDINGS APPENDIX

Applicants are aware of no other current appeals or interferences pertaining to the instant invention. Appellants previously filed a Brief on Appeal for this application, but the U.S. Patent and Trademark Office reopened the application for prosecution so as to address a defect with one of its references.

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MONOGRAPHS ON ALTERNATIVE THERAPIES

Saint John's Wort

Mary L. Chavez, PharmD and Pedro I. Chavez, PhD†*

It is widely recognized that alternative therapies have increased in use, and pharmacists are being asked more often to provide information on these products. Alternative therapy products are classified as "dietary supplements," according to the Dietary Supplement Health and Education Act of 1994 (DSHEA). In section 3 of DSHEA, a dietary supplement is defined as a product intended to supplement the diet and contains one or more of the following: a vitamin, a mineral, or an herb or other botanical or amino acid. According to section 4, these products are excluded from the regulatory approval by the Food and Drug Administration. It is the goal of this feature to provide a critical and unbiased evaluation of alternative therapy products.

Generic Name:
ST. JOHN'S WORT
Synonyms:
HYPERICUM
Proprietary Names:[1]
Fresh Freeze-dried St. John's Wort (Eclectic Institute Inc.)
Hypericalm (Enzymatic Therapy)
Hypericum
(Hypericum Buyer's Club)
Kira (Lichtwer Pharma U.S., Inc.)
Mood Support: St. John's Wort
(Natrol)
St John's Wort (Life Time, KAL, Nature's Resources, Nature's Sunshine, Nature's Way, Solaray, others)
St. John's Wort Extract
(Enzymatic Therapy)
St. John's Wort Tea Bags
(Select Herb Tea Company)
St. John's Wort Whole Extract
(Elixir Tonics & Tea)
Combination Products:
Mood Support Syrup
(Nature's Apothecary)
Nutri Zac (Nature's Plus)
St. John's Solution
(Quantum, Inc.)
St. John's Wort Plus
(Enzymatic Therapy)
Stress Relief
(Nature's Apothecary)
Tension Tamer
(Celestial Botanica)
Proprietary Names in Other Countries:[1,2]
Esbericum, Extract Z 90017, Hyperforat, Lophakomp-Hypericum, Jarsin, Jarsin 300, Neuroplant, Psychotonin M
Combination Products:
Castrophan, Cefaktivon "novum", Cheiranthol, Chelidonium-Strath, Cicaderma, Elisir Depurativo Ambrosiano, Galenavowen-N, Gastritol, Gastrol S, Gutnacht, Hepaciem, Hepaticum-Divinal, Hewepsychon Duo, Hyperforat, Hyperforat-forte, Inconturina, Kalantol-A, Kalantol-B, Kneipp Krauter Taschenkur Nerven und Schlaf N, Kneipp Magentrost, Kytta-Sedativum, Losapan, Marianon, Menstrualin, Migranex, Nephropur, Nerven-Tee Stada, Neurapas, Neuro-Presselin, Oxacant N, Pan-Nerventonikum, Phonix Kalophon, Phytoberidine, Phytogran, Psychatrin, Rhoival, Salus Nerven-Schlaf-Tee Nr.22,

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ALTERNATIVE THERAPIES INDEX

Salusan, Sedariston, Sedariston Konzentrat, Sinedyston, Spray 1000, Ucee, Venacton, Vollmers praparierter gruner, Warondo-Wundsalbe, Worishofener Nervenpflege Dr. Kleinschrod.

St. John's wort is also known as: **amber touch-and-heal, chassedi-able, devil's scourge, goat weed, God's wonder plant, grace of God, klamath weed, millepertuis, rosin rose, and witches' herb.**[1-6]

St. John's wort is a shrubby, aggressive, perennial weed that grows along dry, gravelly roadsides, meadows, woods, hills, and hedges.[1] Normally, the plant grows to a height of 1 to 2 feet, but is reported to grow to 5 feet on the Pacific coast.[4,7] It has glabrous (hairless) erect stems that branch out at the top of the plant. The leaves are pale-green opposite ovals 1 to 2.5 cm long and punctuated with numerous, scattered, small, reddish-black dots (oil glands). The dots are visible to the eye and appear yellowish and translucent when held against the light.[5,8]

The plant has numerous 1.5- to 2.5-cm-wide, five-petaled, star-shaped yellow flowers that have black dots near the margins and pointed green calyces. The flowers have numerous stamens, which occur in three to five brown clusters, and pear-shaped ovaries that have three long, wide styles. The flowers contain a red pigment that produces a red stain when rubbed between the fingers.[7]

St. John's wort blooms profusely between June and September. Flowering is followed by formation of numerous small, cylindric, pitted brownish-black seeds, contained within three-celled resinous capsules.[1,9]

The harvesting period is from July to August. The plant must be dried immediately after harvesting to avoid degradation of the active principles.[9]

CHEMISTRY/CLASS

The chemical constituents of St. John's wort are complex, numerous, and diverse. The amount of the constituents is related to the harvesting period, the drying process, and storage.[3,6,7,9,10] The above-ground plant parts contain tannins (up to 16%), which may be responsible for the plant's wound-healing effects.[1] In addition, the plant contains reddish-colored napthodianthrones, mainly hypericin (up to 0.5%) and its hydroxy derivative pseudohypericin, reported to have antidepressant and antiviral activities.[2,9] Other constituents include flavonoids, essential oils, proanthrocanidins, and other compounds.[6]

A variety of flavonoids are found in the flowers and stalks; these include hyperin/hyperoside, quercetin, isoquercetin, quercetrin, isoquercetrin, amentoflavone, rutin, and biapigenins.[6] The proanthocyanidins consists of (+)-catechin and (-)-epicatechin.[3,4,6,10] The essential oils are mainly monoterpenes, including pinenes, myrcene, limonene, and the sesquiterpenes caryophyllene and humulene.[3,6,9,10] Other compounds include amino acids, beta-sistosterol, carotenoids, choline, isovalerianic acid, lauric acid, myristic acid, nicotinic acid, palmitic acid, pectin, phlobaphene, rhodan, scopoletin, stearic acid, umbelliferone, and xanthones.[3,6,10]

St. John's wort extract ("hypericum") is prepared by treating the plant with mixtures of ethanol and water and then standardizing (prepared for a defined concentration) for the hypericin and hypericin-like constituents.[9] An oil preparation of John's wort ("Hyperici Oleum," "St. John's oil")[11] is typically prepared by extracting the flowers with olive oil.[6] The oil preparation is hypericin-free and contains lipophilic compounds, including sufficiently high concentrations of hyperforin.[11] It can be used internally but most often is used externally for wounds and hemorrhoids.[9,10] The olive oil extract turns a reddish color after exposure to sunlight for several weeks.[4]

HISTORY

There are several theoretic explanations for the plant's name. One is that the name was derived from the belief that the red spots on the leaves were the blood of St. John the Baptist; the spots were thought to appear on the anniversary of the saint's beheading, said to be August 29.[3,7]

Another theory is that the plant blooms its brightest on June 24, which is considered the birthday of St. John the Baptist.[4,12] Others quote a legend that sleeping with a piece of the plant under one's pillow causes St. John the Baptist to appear in a dream. The saint would give his blessing and would prevent the dreamer or a loved one from dying in the upcoming year.[3,13]

Another explanation is that the name resulted from the English tradition of throwing the flowers into a bonfire on the eve of St. John's Day.[6] There is another legend, from the sixth century, that the missionary St. Columbo always carried a piece of the plant as a token of his respect for St. John.[6]

Others report that the name comes from the word "hypo" for under and "erukn" or "ereiken" for heather—meaning that the plant grows under heather. The name wort is derived from the Old English word for plant or herb.[12]

St. John's wort belongs to the family *Hypericaceae.* The official name of the plant is *Hypericum perforatum L.*[6] It is thought that this name was derived from the Greek word yper for "upper" and eikon for

"image," which translates to "over an apparition" or "over an icon."[8,9] It is said that the plant was placed over religious icons as protection against evils spirits, witches, or other supernatural beings. Supposedly they would take one "whiff" of the unpleasant-smelling plant and would immediately depart.[1,7,9,11] The name perforatum, Latin for "perforated," refers to the perforations that appear on the leaves when held up to light.[9]

USE

St. John's wort has been used since Greek and Roman times as a nerve tonic for mood and temperament.[9] Dioscorides, Galen, Hippocrates, Pliny, and Theophrasus wrote about the medicinal properties of the plant.[6] Other folkloric uses include treatment of bronchial inflammation, burns, cancer, enuresis, gastritis, hemorrhoids, hypothyroidism, insect bites and stings, insomnia, kidney disorders, scabies, and wounds.[4,7,9,10,11]

St. John's wort is a popular herbal remedy that is extolled as an alternative to synthetic antidepressants and is currently proclaimed to be "nature's Prozac."[3,10,12] It is widely used in Europe for treatment of mild to moderate depression. St. John's wort is licensed in Germany for the treatment of depression, anxiety, and insomnia[1] and is considered the drug of choice for common depression.[11]

Sales of St. John's wort in Germany outnumber all other antidepressants combined and the herbal remedy outsells Prozac more than seven to one.[11] German physicians prescribed 66 million daily doses of St. John's wort for the treatment of depression in 1994[12]; sales totaled $23 million.[16] In 1996, German sales increased to $66 million.[16] Other uses of St. John's wort include therapy against viral and bacterial infections.[6] St. John's wort is also used in homeopathic remedies.[10]

CLINICAL PHARMACOLOGY

St. John's wort has been extensively studied for its antidepressant effects. Other areas of research include antiviral activity, wound healing, and antineoplastic activity.

Antidepressant Effects

The exact mechanism of how St. John's wort exerts its antidepressant effect is not fully understood. Early studies demonstrated that hypericin inhibited monoamine oxidase (MAO).

Using a commercially available hypericin (80% pure), researchers demonstrated that hypericin inhibited both type-A and type-B MAO in rat brain mitochondria.[18] The sensitivity of type-A MAO was higher than type-B.

Newer in vitro investigations found that hypericin is not the active MAO-inhibiting constituent, but flavonols and 1,3,6,7-tetrahydroxyxanthrone that were present in the remaining 20% of the preparation accounted for the inhibition of type-A MAO.[6,19,21-23] A 95% pure preparation of hypericin did not inhibit type-A or type-B MAO at concentrations up to 10 mcM. The test fractions that contained hypericin and its relatives did not demonstrate relevant MAO-inhibiting activity in sufficient concentrations.[21]

Newer in vitro research suggests that St. John's wort acts as a serotonin reuptake inhibitor.[6,7,9] Hypericum extract in high concentrations modulates the expression of serotonin receptors[9] by reducing their availability, resulting in impaired serotonin uptake in crude rat synaptosomes.[6] However, the high concentration used in the study[9] could never be achieved in whole animals.[6] Others have found that there is an increase in urinary metabolites of serotonin and norepinephrine after treatment with hypericin.[11]

Another possible explanation for the antidepressant activity is the ability of St. John's wort to modulate the production of cytokines. Thiele and Ploch[24] demonstrated that hypericum extract suppressed the release of interleukin-6, an inflammation mediator, in two out of four depressed patients.

It has been hypothesized that interleukins are involved in depression.[25] One theory is that increased levels of interleukins induce depression indirectly in susceptible people.[6] Researchers have demonstrated that interleukin-1 and, more significantly, interleukin-6 have direct activating effect on the hypothalamic-hypophyseal-adrenal axis, causing release of corticotropin-releasing hormone (CRH).[26] Oversecretion of hypothalamic CRH has been found in the cerebrospinal fluid of certain depressed patients. In addition, the number of central CRH receptors is reduced in depression.[26]

Other proposed antidepressant mechanisms include St. John's wort's abilities to inhibit catechol-O-methyltransferase activity and to inhibit receptor affinities for adenosine, benzodiazepine, GABA-A, GABA-B, and inositol triphosphate.[6] Additional studies are needed to determine the exact pharmacological mechanism(s) of the antidepressant effect.

Antiviral Effects

Hypericin and pseudohypericin inactivate a broad range of viruses and retroviruses.[6,27,28-35] There are two proposed mechanisms for the antiviral activity.[28] One mechanism is direct viral inactivation of mature and properly assembled viruses, probably due to interference with the release of active enzymes. The second proposed mechanism is that these compounds interfere with proper assembly or processing of intact virions from infected cells.[28]

Studies suggest that lipid-enveloped viruses and retroviruses are inactivated by hypericum, where-

as unenveloped viruses such as adenoviruses and polio viruses are resistant to hypericin.[30] Hypericin and pseudohypericin eradicate the appearance of reverse transcriptase (RT) in cell lines infected with radiation leukemia virus (RLV).[3] A single 50 mcg dose of both hypericin and pseudohypericin administered concomitantly with, or a day after, inoculation with Friend leukemia virus prevented disease or death in mice.[31]

Hypericin has been shown to possess activity against human immunodeficiency virus type 1, herpes simples virus type 1 and type 2, murine cytomegalovirus, para-influenza 3 virus, Sindbis virus, and vesicular stomatitis virus.[29,31-33] Current research interest focuses on the use of hypericin for the treatment of AIDS, since hypericin prevents HIV invasion of CD4 cells.[29] In addition, researchers have shown that HIV and other viruses present in red blood cells were destroyed by hypericin without causing visual damage to the cells. It has been suggested that hypericin may be useful as an inactivator of retroviruses in blood products.[34,35]

Studies have demonstrated that the virucidal activity is enhanced in a dose-dependent manner by visible light.[3,9,33,34-39] Hypericin produces singlet oxygen and possibly various kinds of radicals in light. Research has demonstrated that photoactivation of hypericin using wavelengths between 400 and 700 nm generated singlet oxygen in mitochondria and inhibited mitochondrial succinoxidase. It is hypothesized that this effect is consistent with a type II singlet oxygen-mediated mechanism for photosensitization.[40]

Wound Healing

The wound-healing properties attributed to St. John's wort may be due to the high content of tannins in the plant, which act as an astringent and have protein-precipitating action. Tannins are present in all aboveground parts of the plant (10% of the whole herb), with the highest concentrations in the flowers (16%).[6,10]

Studies have shown that the sesquiterpenes caryophyllene and humulene possess antifungal activity.[3,10,41] Another study found that a resin fraction of the alcoholic extract of St. John's wort had activity against gram-positive organisms.[6] The tannins and flavonoids inactivate *Escherichia coli* and a commercial product containing 0.412% quercetin was effective against *Staphylococcus aureus* infection.[6]

Antineoplastic Activity

Hypericin and pseudohypericin inactivate protein kinase C in vitro.[3,41] Hypericin was shown to inhibit the growth of glioma cell lines and was found to be a potent inducer of glioma cell death due to inhibition of protein kinase C.[6] In addition, hypericin inhibits the growth of lung cancer and skin cancer in vitro.[13] Recently, investigators have used hypericin in combination laser therapy for photodynamic treatment of cancer.[13]

Other Effects

Other reported effects of St. John's wort include enhancement of coronary flow, inhibition of receptor tyrosine kinase activity, inhibition of release of arachidonic acid and leukotriene B4, and increase in the production of nocturnal melatonin.[6]

PHARMACOKINETICS

The pharmacokinetics of hypericin and pseudohypericin were studied in healthy male subjects using single (n = 12) and multiple doses (n = 13) of a standardized hypericum extract LI 600 (Jarsin 300).[42] Median elimination half-life of hypericin was 24.8 to 26.5 hours for hypericin and 16.3 to 36.0 hours for pseudohypericin. With repeated dosing, steady state occurred in 4 days. The mean total clearance rates were 9.2 mL/min for hypericin and 43.3 mL/min for pseudohypericin. Systemic bioavailability was 14% for hypericin and 21% for pseudohypericin.

The authors suggested that food may interact with the absorption of hypericin and pseudohypericin, which may account for their low bioavailability.[43]

CLINICAL TRIALS/ INVESTIGATIONS

Clinical trials have primarily evaluated St. John's extract for the treatment of depression. Other studies have tested the extract for antiviral activity, protein kinase C inhibition, wound-healing effects, enhancement of coronary flow, augmentation of melatonin production, as a treatment for chronic tension headaches, for hepatoprotective effects, and for the ability to increase bile duct flow.[6]

Antidepression Studies

There are numerous case reports and clinical trials evaluating St. John's wort for mild-to-moderate depression; these studies have included more than 5,000 patients.[11] There have been 25 controlled double-blind studies involving more than 2,000 patients. Response rates have been mainly between 50% and 80%.

A meta-analysis of 23 randomized trials of St. John's wort in the treatment of depression was recently published.[2] All clinical trials used preparations that were standardized for hypericin content. The 23 randomized clinical trials included 1,757 outpatients with mild to moderately severe depression. There were 15 trials (n = 1,008) that were placebo-controlled (Table 1), and 7 trials (n = 749) that compared hypericum to other antidepressants (Table 2). Duration of treatment was from 4 to 12 weeks.

Thirteen trials provided informa-

tion on treatment response when hypericum was compared to placebo. A total of 55.1% of patients were considered to be "treatment responders" with hypericum compared with 22.3% with placebo. Hypericum as a single preparation was compared to standard antidepressant drugs. There were 63.9% treatment responders with single hypericum preparations, and 58.5% treatment responders with standard antidepressant drugs.

Combination preparations with hypericum were compared with standard antidepressant drugs, and the results indicate that the combination preparations were slightly better than single preparations. A total of 67.7% of patients responded to the combination products compared with 50% of those given standard antidepressants. Adverse effects were less frequent with hypericum than with antidepressants. The authors concluded that hypericum extract was more effective than placebo and equally effective as standard antidepressants.

Patients who suffer from seasonal affective disorder (SAD) develop depression during the autumn and winter months and have full remission in spring and summer (DSM-IV).[44] A small trial investigated the effect of hypericum on SAD.[45] Patients (n = 20) with SAD were divided into two groups.

All patients received 900 mg of St. John's wort extract (standardized to 0.3% hypericin). However, one group received the standard treatment for SAD of bright lights (3000 lux light, which corresponds to looking out the window on a bright spring day),[13] and the other group received dim light (< 300 lux light) for 2 hours daily. Dim light should not be beneficial for patients with SAD.

Both groups demonstrated improvement. No adverse reactions were reported in either group. The authors suggested that the antidepressant effect of St. John's wort may be beneficial in patients with SAD.[45] The results of this trial are questionable since the trial involved a very small number patients, which could account for the improvement in outcome measurements in the two groups; additional studies are needed.

Antiviral Studies

Research interest has focused on the antiviral activity of hypericin in hopes of finding a new treatment for HIV infection. The FDA has sanctioned hypericin as an investigational new drug. Trials have been conducted using VIMRxyn®, a synthetic hypericin manufactured by VIMRx Pharmaceuticals.[4]

There have been two AIDS Clinical Trial Group (ACTG) studies that used synthetic hypericin as a treatment for AIDS. The ACTG 150 trial was completed in February 1995, and the ACTG 258 Phase I/II trial was withdrawn on November 2, 1995.[4] Both trials had problems related to photosensitivity reactions.

Results from two other clinical trials were reported at the International Conference on AIDS held in 1996.[47,48] An open trial assessing the long-term efficacy of hypericin was conducted in 18 patients with AIDS that were seropositive and symptomatic. Patients received hypericin intravenously as 2 mL weekly plus 12 tablets of hypericum perforatum tablets (of undefined dosages) daily for periods of 48 to 72 months. Among the 18 participants, six had pretreatment concentrations of serum p24 antigen, one became positive after 24 months, 12 remained p24 negative throughout, four exhibited significant long-term declines in p24 antigen levels, and two remained unchanged. A substantial decline in viral load was observed in most patients with p24 antigen.

Another study that was presented at the International Conference on AIDS was a trial conducted in Thailand, which evaluated the maximum tolerable oral dose of synthetic hypericin.[48] The study was an open-label, sequential-dose-escalation tolerance study in two groups of four HIV-positive subjects.

The first group received oral hypericin as 0.05 mg/kg (average dose 2.9 mg). Three patients completed the 28 days of treatment; one patient received the drug for 17 days only. Three of the four subjects developed mild photosensitivity reactions on exposure to sunlight.

The second group was given 0.16 mg/kg (average dose 9.2 mg). Two patients developed intolerable photosensitivity reactions and the drug was discontinued. Two others developed mild but tolerable photosensitivity reactions, which caused the researchers to reduce the drug to 0.05 mg/kg after 7 to 10 days to complete the 28-day treatment period.

A follow-up Phase I/II clinical trial was conducted in Thailand.[49,50] Hypericin was administered once daily for 28 days to 12 HIV-infected patients at a dose of 0.05 mg/kg. No photosensitivity was noted. Based on reduction in viral load (quantitated using polymerase chain reaction of plasma), changes in serum HIV RNA values ranged from no change to a 97% reduction, with a trend for larger decreases in patients with a higher baseline viral load. Ten of 12 patients had evidence of anti-HIV activity.

Other Studies

VIMRx has recently completed a Phase I clinical trial to evaluate VIMRxyn as a topically applied phototherapy for skin disease including psoriasis, cutaneous T-cell lymphoma, warts, and Kaposi's sarcoma. Phase I/II clinical trials will be conducted to determine the antiviral effectiveness of VIMRxyn in reducing viral load in patients with infectious chronic hepatitis C.[51]

TABLE 1

Trials Comparing Hypericum With Placebo for Treatment of Depression (Grouped by Preparation)

Preparation	*Number of Trials*	*Total Number of Patients in Trials*	*Total Hypericin/ Day (mg)*	*Total Plant Extract/ Day (mg)*	*Duration (weeks)*	*Parameters Measured*	*Response Rate*
Extract Z 90017	1	112	1-2	500-1,000	6	Bf-S	53%
Hyperforat	1	60	0.6	NR	6	Own scale	15%
Jarsin (tablets)	3	165	1.08	900	4-6	HAM-D, B-L, CGI KAI, STAI	12%-33%
Jarsin 300 (tablets)	3	217	2.7	900	4	HAM-D, BEB, BL, CGI	28%-81%
Neuroplant (capsules)	1	50	1	500	8	HAM-D	45%
Neuroplas	1	60	0.48	600	8	D-S	0%
Psychotonin M (drops)	5	344	0.5%-0.75%	350-500	4	HAM-D	23%-49%

*Combination preparation of hypericum plus 50 mg valeriana

BEB = Hansgens Complaint Inventory; Bf-S = von Zerssen's Self-Rating Scale; B-L = von Zerssen's Health Compliance survey; CG I= Clinical Global Impression Score; D-S = Depression Scale according to von Zerssen; KAI = Lehrl's Short Test on General Information Processing; SDS = Self Rating Depression scale; STAI = State Trait Anxiety Inventory; NR = not reported

Sources: Linde K et al[1]; Upton R[4]; Bombardelli E, Morazzoni P[5]

DOSAGE

The European Scientific Cooperative for Phytomedicine (ESCOP) recommends a daily dose of 0.2 to 1 mg total hypericin for treatment of depression.[13] Others recommend a minimum daily dose of 1 mg hypericin.[7] Most clinical trials used standardized extracts with 0.3% hypericin, which is approximately equivalent to a daily dose of St. John's extract of 300 mg three times daily.[7] There is confusion on how to dose hypericum. The current recommendation is that the dose be based on the total plant extract and not just the hypericin content.[9,13]

TOXICITY

This plant is relatively free from harmful activity, although there are potential problems. Hypericin is believed to cause photosensitivity to individuals who use the drug for long periods of time and then are exposed to the sun. In the 1970s, the Food and Drug Administration (FDA) classified St. John's wort as "unsafe" because of reports of grazing animals developing phototoxicity after eating large amounts of the plant.[7]

Phototoxicity with hypericum in range animals is well documented.[6] Photosensitivity in livestock is referred to as hypericism or "light sickness."[9] Reported reactions are mainly dermatological, such as severe erythema and edema of skin, conjunctiva, and buccal mucous membranes, which can lead to restlessness, psychomotor excitement, blindness, and refusal to eat by the animals.[6] When the erythema and edema subside, the affected skin becomes dry and necrotic. The photodynamic action is attributed primarily to hypericin. Hypericin must be ingested to cause photosensitivity and does not cause it by contact. It does not affect any other organ other than the skin.[6]

St. John's wort has a long history of use in Europe and some references indicate that there are no reports of phototoxicity in humans.[9,12] A study using cell cultures of human keratinocytes found that phototoxicity occurred only when the therapeutic dose was exceeded by 30- to 50-fold.[9] No photodynamic effects were observed in the study that determined the pharmacokinetics of hypericin and pseudohypericin.

However, in the previously cited study of AIDS patients, photosensitivity was reported.[47] The study used hypericin oral doses of 0.05 mg/kg and 0.16 mg/kg. Most patients (three out of four) developed photosensitivity in the 0.05 mg/kg group. All patients in the 0.16 mg/kg group developed photosensitivity, and the

TABLE 2

Trials Comparing Hypericum to Antidepressants for Treatment of Depression (Grouped by Preparation)

Test Preparation	*Daily Dose of Anti-depressant*	*Number of Trials*	*Total Number of Patients in Trials*	*Total Hypericin/ Day (mg)*	*Total Plant Extract/ Day (mg)*	*Duration of Trial (weeks)*	*Parameters Measured*	*Response Rate*
Compared with Amitriptyline:								
Esbericum	30	1	80	0.75	NR	6	HAM-D, BF-S	38%
Sedariston*	75-100	1	162	0.45-0.9	300-600	6	HAM-D, B-L, CGI	80%
Compared with Bromazepam:								
Psychotonin M (drops)	6	1	80	0.75	500	4	HAM-D	NR
Compared with Desipramine:								
Sedariston*	100-150	1	100	0.6-0.9	400-600	6	B-L, CGI, DS	30%
Compared with Diazepam:								
Hyperforat	6	1	60	0.4	NR	12	HAM-D, CGI, SDS	50%
Compared with Imipramine:								
Jarsin 300 (tablets)	75	1	135	2.7	900	6	HAM-D, CGI, D-S	62%
Psychotonin M (drops)	50	1	30	0.75	500	2	HAM-D	60%
Compared with Maprotiline:								
Jarsin 300 (tablets)	75	1	102	2.7	900	4	HAM-D, CGI, D-S	67%

*combination preparation with Hypericum plus *Corydalis cavae, Eschscholtziae californiae,* Valeria.

BEB = Hansgens Complaint Inventory; Bf-S = von Zerssen's Self-Rating Scale; B-L = von Zerssen's Health Compliance Survey; CGI = Clinical Global Impression Score; D-S = Depression Scale according to von Zerssen; KAI = Lehrl's Short Test on General Information Processing; NR = not reported; DS = Self-Rating Depression Scale; STAI = State Trait Anxiety Inventory

Sources: Linde K et al[2]; Upton R[4]; Bombardelli E, Morazzonni P[9]

drug had to be discontinued in two of four patients and reduced in two of four patients.

There is a report from Germany of a 61-year-old woman who developed recurring, elevated, itching erythematous lesions in light-exposed areas after taking St. John's wort extract for 3 years (unknown dose).[13] Although photosensitivity appears to be rare at recommended doses, patients should be warned about possible photosensitivity, especially in fair-skinned patients and those receiving concurrent photosensitivity-producing drugs.

The incidence of adverse reactions reported in clinical trials ranged from none to 25%.[14] The most common reported adverse effects were emotional vulnerability, fatigue, pruritus, and weight increase.[7] Other minor side effects that have been reported are gastrointestinal discomfort, fatigue, dry mouth, and dizziness.[12]

In an open study of 3,250 patients that lasted 4 weeks, 2.4% of patients developed adverse reactions and 1.5% dropped out of the study.[15] The most common adverse effects were gastrointestinal (0.6%), allergic reactions (unknown type) (0.5%), fatigue (0.4%), and restlessness (0.3%).[15] Some references advise that it is especially important to monitor patients during the first 2 to 4 weeks of therapy.[7,13]

Because extracts containing quercetin have been found to possess

cytotoxic and mutagenic activity in both in vivo and in vitro test systems, the safety of St. John's wort has been questioned.[52] Still other researchers found that St. John's wort has antimutagenic activity on UV-induced beta-galactosidase in cells.[56]

In addition, quercetin is abundantly found in many plants. It is estimated that the average person consumes 50 mg of quercetin and related flavonoids daily from fruit and vegetables. The use of St. John's wort in recommended amounts would increase the daily consumption of quercetin by only 1 mg, so concern about safety is probably unwarranted.[52]

CONTRAINDICATIONS

St. John's wort should not be used in combination with tyramine-containing foods, alcoholic beverages, narcotics, amphetamines, and over-the-counter cold and flu medications. The presence of MAO inhibitors may also indicate limiting the use of foods such as cheese, pickled meat, and wine. St. John's wort should not be used in patients receiving selective serotonin reuptake inhibitors. There is insufficient information on the safety of using St. John's wort during pregnancy; it is therefore not recommended in pregnant women.[3]

CONCLUSION

St. John's wort has a long history of use in Europe. A large number of clinical trials support its use for mild-to-moderate depression. It appears to have a favorable adverse effect profile. St. John's wort is less expensive than most antidepressants. The typical daily dose of Prozac is $2.50 per day compared with $0.30 per day for Hypericum.[16]

An editorial in the *British Medical Journal* accompanying the meta-analysis of the clinical trials concluded that the data is promising, but there is still not enough evidence to support the use of St. John's wort as an effective antidepressant preparation.[57]

The meta-analysis noted limitations in the clinical trials that made interpretation of the results difficult.[2] None of the trials lasted longer than 12 weeks. Most of the trials were small and used heterogenous groups of patients. Classification of depression was inconsistent, and symptoms did not correlate with depression scales. There was a lack of intent-to-treat analysis. Daily doses in the trials varied and ranged from 0.4 to 2.7 mg/day of hypericin. Dosage of antidepressants in the comparison trials were in the low range.

Additional clinical trials using better methodology comparing hypericum with antidepressants are needed. The types of depression evaluated need to be better delineated, and all types of depression including severe forms should be evaluated. Long-term studies are needed to assess the emergence of side effects and the risk of relapse.

On June 6, 1997, the National Institutes of Health's Office of Alternative Medicine, the National Institute of Mental Health, and the Office of Dietary Supplements announced that they will collaborate to fund research to determine the efficacy of a standardized extract of St. John's wort for the treatment of depression.[58] Although promising, additional trials are needed to evaluate the role of St. John's wort as an antiviral agent.

The serious nature of self-treating depression is of concern. St. John's wort should not be considered an over-the-counter medication for self-diagnosed depression. Patients should be advised to see a qualified health professional before deciding to use St. John's wort.

ADDENDUM

Since the withdrawal of Redux® and fenfluramine, St. John's wort has been promoted as a herbal alternative to "fen-phen" for weight loss.[59] The dietary supplement is touted as an appetite suppressant because of its ability to increase serotonin levels.

Herbal fen-phen products usually contain St. John's wort and mahuang (or ephedra). Diet-Phen® (Source Naturals) is a combination of St. John's wort 900 mg with ephedra 12 mg, L-phenylalanine 500 mg, chromium 200 mcg, and a small amount of niacin and vitamin B-6/dose. Herbal-Phen-Fen® (developed by Nutri/System and distributed by HPF) contains St. John's wort extract (0.3% hypericin) and mahuang extract (8% alkaloids)/dose.

These combination products could potentially cause significant adverse effects. Ephedra is a potent sympathomimetic and should not be used in combination with serotonin reuptake inhibitors or monoamine oxidase inhibitors (both possible effects of St. John's wort). The FDA has received about 800 reported adverse events with other ephedra-containing products since 1993, including 38 deaths.[59] On June 24, 1994, the FDA proposed legislation for the safe use of dietary supplements containing ephedrine alkaloids.[60]

Combination products containing St. John's wort and ephedra should not be recommended. There are no clinical trials supporting the use of St. John's wort as a weight-loss supplement.

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Managed Care

Continued from page 1608

before it is applied in any one society. Recognition of societal needs and wants balanced with fiscal sense is a common theme in all countries when dealing with health care system issues. Creating a solution for the United States requires not only political will to enact appropriate measures and administrative apparatus, but also a dedication to certain values.

Health care in all societies is a social system and not merely a personal service. Health care evolved from religious beliefs in all countries, not from commerce. The United States can continue to endorse self-interest in other economic markets, but it cannot resolve its difficulties in health care without institutionalizing social solidarity around a health system.

A mixture of financing means can be gleaned from European systems and put into place if the will to enact and follow those measures is shown by the American people. The experiences of other countries, in both success and failure, can provide invaluable lessons to those who want to reform the U.S. health care system—including the profession of pharmacy.

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55. New Hyperforin Derivatives from *Hypericum revolutum* VAHL with Growth-Inhibitory Activity against a Human Colon Carcinoma Cell Line[1])

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(8.II.89)

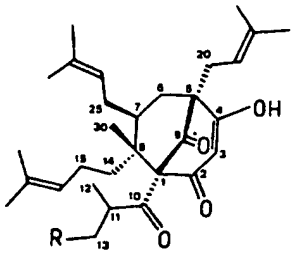
The crude petroleum-ether extract of the root bark of *Hypericum revolutum* VAHL (Guttiferae) exhibited *in vitro* growth-inhibitory activity against the Co-115 human colon carcinoma cell line. Activity-guided fractionation of this extract resulted in the isolation of two new hyperforin derivatives **1** and **2**. The structure of **1** (hyperevolutin A) was established by X-ray analysis as the 4-hydroxy-8-*exo*-methyl-5,7-*exo*-bis(3-methylbut-2-enyl)-1-(2-methyl-1-oxopropyl)-8-*endo*-(4-methylpent-3-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione. The structure of the homologue **2** was deduced by comparison of its UV and ^{1}H- and ^{13}C-NMR spectra with those of **1**.

Introduction. – The antibiotic properties of extracts of *Hypericum* species (Guttiferae) are well established [1–3]. One active constituent, designated hyperforin, was isolated from *Hypericum perforatum* L. and first characterized in 1971 [4] [5]. A number of reports have appeared on this novel antibiotic, particularly on its controversial absolute configuration [6–9], but finally X-ray analysis of its 3,5-dinitrobenzoate [10] and *p*-bromobenzoate [11] allowed direct proof of its absolute configuration.

Recent phytochemical investigations of various *Hypericum* species for new bioactive products led to the isolation of saroaspidine A, B, and C from *Hypericum japonicum* THUNB. [12] and of chinesin 1 and 2 from *Hypericum chinense* L. [13]. All isolated compounds were shown to display interesting antimicrobial activities against various microorganisms. In addition, chinesin 1 had relatively strong activity in a cytotoxicity test against *HeLa* cells.

Finally, in the course of our systematic chemical and biological screening studies of African plants, antifungal chromenyl ketones have been isolated from the aerial parts of *Hypericum revolutum* VAHL [14]. *Hypericum revolutum* is a shrub native to South-East Africa, growing at altitude along the margins of evergreen forests. More extensive investigations showed that the petroleum-ether extract of the root bark of *Hypericum revolutum* displayed significant growth-inhibitory activity against the Co-115 human colon carcinoma cell line. Hyperevolutin A (**1**) and B (**2**) were shown to be the main active

[1]) Presented in part at the 36th Annual Congress on Medicinal Plant Research, Freiburg im Breisgau, FRG, 12–16th September, 1988, and at the Meeting of the Swiss Chemical Society, Bern, 21st October, 1988.



1 R = H[2])
2 R = CH_3(31)[2])

components [15]. Both compounds appeared to be related to the antibiotic hyperforin isolated from *Hypericum perforatum* L.

Results. – The root bark of *Hypericum revolutum* collected in Malawi was extracted with petroleum ether. This extract showed growth-inhibitory activity against Co-115 colon tumour cells (ED_{50} = 3.4 µg/ml). In order to localize the activity, the light petroleum-ether extract was subjected to bioassay-guided fractionation by droplet countercurrent chromatography (DCCC) with a H_2O-containing solvent system (see *Exper. Part*) giving an enriched fraction (ED_{50} = 1.4 µg/ml) which contained a compound that was crystallized from hexane. The crystalline product was the major active constituent of the extract with an ED_{50} of 0.7 µg/ml and was subjected to single-crystal X-ray analysis.

Although the result of the X-ray analysis suggested structure **1** with a mol. wt. of 468, the DCI-MS (reactant gas NH_3) of the crystalline material showed the presence of peaks at *m/z* 483 and 500, in addition to the expected peaks at *m/z* 469 ($[M + H]^+$) and 486 ($[M + NH_4]^+$). This difference of 14 amu suggested the presence of a higher homologue of **1** with a mol. wt. of 482. Anal. HPLC on *RP-18* using a photodiode-array detector showed the crystals, active in the Co-115 assay, to be a mixture of two compounds with identical UV spectra. A base-line separation of the two products occurred only after addition of 0.1% AcOH to the MeOH/H_2O mixture (87:13). Semi-prep. HPLC of the

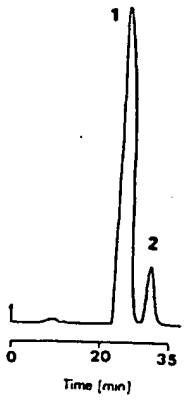


Fig. 1. *Semi-prep. HPLC of hyperevolutin A* (**1**) *and B* (**2**). *µBondapak C18* (30 cm × 7.8 mm i.d.); MeOH/H_2O 83:17 (0.1% AcOH added to the solvent mixture); UV detection at 275 nm; flow-rate 5 ml/min; 120-mg sample in 2.4 ml of MeOH; injection of 150-µl portions.

[2]) Arbitrary numbering, see *Fig. 2*.

crystalline material (MeOH/H_2O 83:17, 0.1% AcOH; see *Fig. 1*) yielded hyperevolutin A (**1**; DCI-MS: 469 ($[M + H]^+$), 486 ($[M + NH_4]^+$)) and a minor compound, hyperevolutin B (**2**), with a mol. wt. of 482. The main compound **1** was the lowest homologue whose ^{1}H- and ^{13}C-NMR and DCI-MS data were in accordance with the structure deduced from X-ray analysis (see *Fig. 2* below, and *Exper. Part*).

The overall geometry of **1** is very similar to that observed for the *p*-bromobenzoate of hyperforin [11]. Both molecules consist of a bicyclic tetraketone in its enol form with three and four side chains, respectively, terminating in an isobutenyl group. As in hyperforin [11], the saturated six-membered ring moiety has a chair conformation. The unsaturated six-membered ring moiety has an envelope conformation with C(9) displaced by 0.60(1) Å from the best least-squares plane through the remaining five atoms. An interesting feature of both compounds is the presence of three carbonyl groups attached to C(1) (see *Fig. 2*), two of which being part of a ring and one of a side chain. Molecules related by the screw axis in the *a* direction are linked by a H-bond involving O*H*–C(4) and C(2)=*O* (see *Table 2, Exper. Part*). There are no further short intermolecular contacts involving non-H atoms.

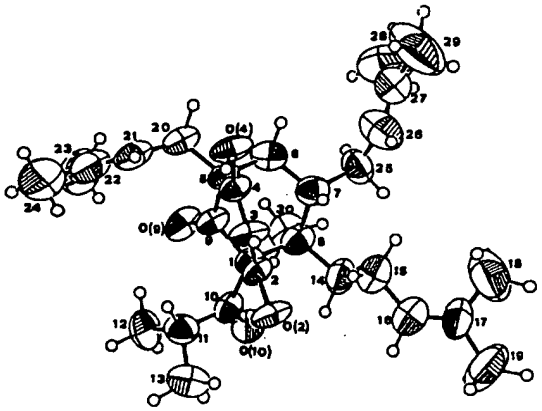


Fig. 2. *View of hyperevolutin A* (**1**) *showing the crystallographic atomic numbering scheme*[2]) *and the vibrational ellipsoids* (50% probability level)

The structure of the closely related compound **2** was deduced mainly by comparison of its ^{1}H- and ^{13}C-NMR spectra with those of **1**.

In the ^{1}H-NMR spectrum, the signals of the isobutyryl group in **1** with 1 *sept.* (J = 6.5 Hz) at 2.31 ppm for H–C(11), 1 *d* at 1.16 ppm (J = 6.5 Hz) for CH_3(12), and 1 further obscured *d* at 1.07 ppm for CH_3(13) were replaced in **2** by those corresponding to a 2-methylbutyryl group in which CH_3(12) appeared at 1.14 ppm (*d*, J = 6.7 Hz) and the terminal CH_3(31) at 0.83 ppm (*dd*, J = 7.4, 7.4 Hz)[2]). Comparison of the ^{13}C-NMR spectra gave further evidence of the structural difference between **1** and **2**. The signals of the isobutyryl group in **1** with 2 CH_3 (21.5 and 20.7 ppm for C(12) and C(13)) and 1 CH group (41.9 ppm for C(11)) were replaced in **2** by characteristic signals confirming the 2-methylbutyryl moiety: 1 CH (48.7 ppm, C(11)), 1 CH_2 (27.6 ppm, C(13)), and 2 CH_3 groups (16.6 and 11.5 ppm, C(12) and C(31), resp.)[2]). As expected, the UV spectra of **1** and **2** were identical as the chromophore is not affected by the introduction of an additional CH_2 group in **2**.

As in the case of hyperforin [7], the UV spectrum of hyperevolutin A (**1**) depended on the pH (see *Exper. Part*) and on the concentration. A $6 \cdot 10^{-5}$ M solution of **1** in MeOH

had a maximum absorption at 273 nm, whereas a more dilute MeOH solution ($2.6 \cdot 10^{-5}$ M) absorbed at higher wavelength (285 nm). These observations indicated the ionogenic nature of its chromogenic grouping which, according to the ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$, should exist predominantly in the enolic form in $CDCl_3$. Preliminary observations in the ^{1}H-NMR spectrum of **1** in dilute $CDCl_3$ solutions suggested the existence of an equilibrium between the enol form **1** and its diketo tautomer.

The ^{1}H-NMR ($CDCl_3$) of a 0.09M solution of **1** showed 1 olefinic *s* at 6.16 ppm for H–C(3) and 1 broad signal at 7.04 ppm, obviously belonging to the enolic OH–C(4). At this concentration, the ^{13}C-NMR spectrum of **1** revealed only the presence of the enolic form with 2 quaternary C-atoms at 187.9 and 185.2 ppm (C(4) and C(2)) and 1 CH group at 109.7 ppm (C(3)). However, careful examination of the ^{1}H-NMR ($CDCl_3$) of a 0.03M solution of **1** showed some concentration-dependant variations which probably could be explained by the presence of the minor diketo tautomer of **1**: 2 small *d* coupled to each other (J = 19.2 Hz) appeared at 3.72 and 3.47 ppm, providing evidence for the geminal CH_2(3) group of the diketo tautomer. Furthermore, the 2 broad *m* (2.51 and 2.48 ppm) of $CH_2(20)^2$) of **1** in a 0.09M solution whose assignments were deduced by comparison with soulattrone **A**, a related bicyclic terpenoid isolated from *Calophyllum soulattri* (Guttiferae) [16], changed into 2 unresolved *dd* on dilution (0.03M).

Discussion. – During our systematic screening of African plants for biologically active substances, two new growth-inhibitory compounds, hyperevolutin A (**1**) and B (**2**), have been isolated from the root bark of *Hypericum revolutum* VAHL. Both compounds possess an enolic proton, thus explaining their irregular behaviour on solid supports, especially silica gel, to which they have the tendency of irreversibly adsorbing. In that respect, the use of a liquid-liquid chromatography technique, DCCC, was essential to isolate these two biologically active compounds [17]. It is shown here that this method is suitable for the fractionation of lipophilic constituents, even with the use of a H_2O-containing solvent system.

The crystalline mixture **1/2** was tested against the Co-115 human carcinoma cell line. The complete details of the biological assay have been given elsewhere [18]. In our test, the activity of the crystalline mixture **1/2** (ED_{50} = 0.7 µg/ml, $1.5 \cdot 10^{-6}$ M reported on the mol. wt. of **1**) was comparable to that of 5-fluorouracil, a synthetic drug used in the therapy of colon cancer (ED_{50} = 0.23 µg/ml, $1.8 \cdot 10^{-6}$ M in EtOH). Tests are underway to evaluate the activity of hyperevolutin A (**1**) on other solid tumor cell lines and to subject the compound to additional bioassays. Preliminary results showed hyperevolutin A and B to have no antifungal activity against *Cladosporium cucumerinum* fungi in a TLC bioassay [19].

Experimental Part

General. TLC: silica gel precoated Al sheets (*Merck*); detection: 254 nm and *Godin* reagent [20]. Droplet counter current chromatography (DCCC): *Büchi-670*-DCC chromatograph, 580 tubes (length 40 cm, 290 tubes with 3 mm i.d. and 290 tubes with 2.7 mm i.d.). Anal. HPLC: *µBondapak C-18* (30 cm × 3.9 mm i.d.; *Waters*); *Spectra Physics 8700* pump; the chromatogram at 275 nm and the UV/VIS spectra were recorded with a photodiode-array detector *HP 1040A (Hewlett-Packard)*. Semi-prep. HPLC: *µBondapak-C-18* column (30 cm × 7.8 mm i.d.; *Waters*); *Waters 6000A* pump coupled with a *Waters* solvent-delivery system (automatic gradient controller); detection at 275 nm with a *LKB 2238 Uvicord S II* detector. M.p.: *Mettler FP 80/82* hot stage apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV spectra: *Perkin-Elmer-Lambda-3* spectrophotometer. IR spectra: *Perkin-Elmer 681*. ^{1}H- and ^{13}C-NMR spectra: *Varian VXR 200* equipped with a switchable 5-mm probe at 200 and 50.1 MHz, resp.; $CDCl_3$ solns.; chemical shifts in δ (ppm) rel. to TMS as internal standard. DCI-MS: *Nermag R 1030* spectrometer.

Plant Material. Root bark of *Hypericum revolutum* VAHL were collected on Zomba plateau, Malawi, in April 1988. A voucher specimen is deposited at the Herbarium, Chancellor College, University of Malawi, Zomba. Another batch of *Hypericum revolutum* was collected in May 1988 near Nelspruit, in E. Transval (South Africa) by the Head of the Lowveld Botanical Garden.

Extraction and Isolation. The powdered root bark (104 g) was extracted at r.t. with light petroleum ether: 3.25 g of extract (ED_{50} = 3.4 μg/ml). A 2.5-g portion was fractionated by DCCC (light petroleum ether/94% EtOH/AcOEt/H_2O 83:67:33:17, ascending mode, flow-rate *ca.* 25 ml/h) to afford 410 mg of the enriched fraction (ED_{50} = 1.4 μg/ml) which was subjected to crystallization from hexane: 205 mg of white crystalline **1/2**. The crystals were subjected to growth-inhibition testing (ED_{50} = 0.7 μg/ml) and to single-crystal X-ray analysis. A 120-mg portion in 2.4 ml MeOH was separated by semi-prep. HPLC (injection volume 150 μl) on a *μBondapak C18* column (30 cm × 7.8 mm i.d., *Waters*) with MeOH/H_2O 83:17 (0.1% AcOH added to the solvent) to afford 110 mg of hyperevolutin A (**1**) and 7 mg of hyperevolutin B (**2**).

The crystalline material obtained from the petroleum-ether extract of the batch of *Hypericum revolutum* collected in South Africa appeared to have a slightly higher content of **2**. Semi-prep. HPLC on a *μBondapak C18* column (30 cm × 7.8 mm i.d., *Waters*) of 120 mg of the crystalline material in 2.4 ml of MeOH (injection volume 200 μl) using MeOH/H_2O 80:20 (0.1% AcOH added to the solvent system) yielded 93 mg of **1** and 17 mg of **2**.

4-Hydroxy-8-exo-methyl-5,7-exo-bis(3-methylbut-2-enyl)-1-(2-methyl-1-oxopropyl)-8-endo-(4-methylpent-3-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione (= *Hyperevolutin A*; **1**). White prisms from hexane. M.p. 128–131°. TLC (SiO_2, light petroleum ether/AcOEt/AcOH 70:30:3): R_f 0.27, orange with *Godin* reagent $[\alpha]_D^{25}$ = +84.4 (*c* = 0.5, MeOH). UV (MeOH, $6 \cdot 10^{-5}$ M): 273. UV (MeOH, $2.6 \cdot 10^{-5}$ M): 285. UV (MeOH, 0.1% AcOH): 269 (11400). UV (MeOH, 0.1% AcOH/$AlCl_3$) and UV (MeOH, 0.1% AcOH/$AlCl_3$/HCl): unchanged. UV (MeOH, 0.1% AcOH/NaOMe): 286. UV (MeOH, 0.1% AcOH/NaOAc) and UV (MeOH, 0.1% AcOH/NaOAc/H_3BO_3): 286. IR (KBr): 3430*w*, 2970, 2920, 2870, 1730, 1600, 1510, 1490, 1450, 1320, 1240. ^{1}H-NMR (200 MHz, $CDCl_3$, 35 mg/0.8 ml[2]): 7.04 (br. *s*, OH–C(4)); 6.16 (*s*, H–C(3)); 5.02–4.99 (*m*, H–C(16), H–C(21), H–C(26)); 2.51, 2.48 (*m*, CH_2(20)); 2.31 (*sept.*, *J* = 6.5, H–C(11)); 2.14, 2.09, 1.95, 1.88, 1.82, 1.76 (br. *m*, 6 H); 1.67, 1.64, 1.56 (3 *s*, CH_3(18), CH_3(19), CH_3(23), CH_3(24), CH_3(28), CH_3(29)); 1.49, 1.44, 1.38 (*m*, 2 H); 1.16 (*d*, *J* = 6.5), 1.07 (obscured *d*, CH_3(12), CH_3(13)); 1.05 (*s*, CH_3(30)); at lower concentration (10 mg/0.8 ml, *ca.* 0.03M, $CDCl_3$): 3.72, 3.47 (2 *d*, *J* = 19.2, CH_2(3) of the minor diketo tautomer). ^{13}C-NMR (50.1 MHz, $CDCl_3$, 35 mg/0.8 ml; multiplicities from DEPTGL experiments): 210.2, 206.6 (C(9), C(10)); 187.9, 185.2 (C(2), C(4)); 135.0, 133.5, 131.3 (C(17), C(22), C(27)); 124.5, 122.2, 119.0 (C(16), C(21), C(26)); 109.7 (C(3)); 80.3, 60.0, 48.3 (C(1), C(5), C(8)); 42.3, 41.9 (C(7), C(11)); 39.4, 37.0, 29.2, 27.8, 24.8 (C(6), C(14), C(15), C(20), C(25)); 25.9, 25.8, 25.7 (C(19), C(24), C(29)); 21.5, 20.7 (C(12), C(13)); 18.1, 18.0, 17.7 (C(18), C(23), C(28)); 14.1 (C(30)). DCI-MS: 486 ($[M + NH_4]^+$), 469 ($[M + H]^+$).

X-Ray Analysis of **1**. Suitable crystals, in the form of transparent blocks, were grown from hexane. Crystal data: $C_{30}H_{44}O_4$, M_r = 468.7, space groupe $P2_12_12_1$, *a* = 9.456(2), *b* = 15.256(4), *c* = 20.445(3) Å, *V* = 2949.4 Å^3, *F*(000) = 1024, *Z* = 4, D_x = 1.055 gcm^{-3}, Mo*Kα*, *λ* = 0.71073 Å, *μ* = 0.38 cm^{-1}. A crystal of dimensions 0.42 × 0.38 × 0.30 mm was used for data collection. Preliminary *Weissenberg* and precession photographs indicated the crystals to be orthorhombic, space group $P2_12_12_1$. Intensity data, with index limits *h* 0 to 10, *k* 0 to 16, *l* 0 to 22 and θ_{max} = 22.5°, were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated Mo*Kα* radiation) using the ω/θ scan mode. There was no significant intensity variation for 4 standard reflections measured every h. Of the 2108 unique reflections measured, 1526 were considered observed ($F_0 > 3\sigma(F_0)$). Cell parameters from ± *ω* values of 20 reflections and their *Friedel* pairs in the range 15° < 2*θ* < 22°. No absorption or extinction correlations applied. The structure was solved by direct methods using the program SHELXS-86 [21]. The program SHELX-76 [22] was used for all further calculations. In the final cycles of least-squares refinement, the hydroxy proton H(O4) was located in a difference map and refined isotropically. The remainder of the H-atoms were included in idealized positions with the CH_3 groups treated as 'rigid groups' (C–H 1.08 Å, H–C–H 109.5°) [22]. Overall isotropic thermal parameters were assigned to the CH and CH_2, the C=CH_2 and the CH_3 protons (refined values 0.091, 0.246, and 0.257). Weighted anisotropic full-matrix least-squares refinement for 1524 reflections (2 reflections probably suffering from extinction were removed) converged at *R* = 0.078, R_w = 0.077; $w^{-1} = \sigma^2(F_0) + 0.00131(F_0^2)$. Average parameter shift/e.s.d. < 0.15. Heights in final difference map ρ_{max} = 0.26, ρ_{min} = −0.21 eÅ^{-3}. Atomic scattering factors were taken from [23]. Final positional and equivalent isotropic thermal parameters are given in *Table 1*, bond distances and angles in *Table 2*. The crystallographic numbering scheme is apparent from *Fig. 2*, prepared using ORTEP-II [24]. Supplementary material is available from *H.St.-E.*

Table 1. *Final Positional and Equivalent Isotropic Thermal Parameters* (× 10^4). E.s.d.'s in parentheses.

$$U_{eq} = 1/3 \sum_i \sum_j a_i^* \cdot a_j^* (\vec{a}_i \cdot \vec{a}_j).$$

Atom	x/a	y/b	z/c	U_{eq} [Å^2]
C(1)	-3(6)	3665(5)	3770(3)	555(26)
C(2)	-397(8)	3035(5)	4316(3)	591(28)
O(2)	601(6)	2682(4)	4615(3)	832(22)
C(3)	-1837(6)	2861(6)	4468(4)	650(30)
C(4)	-2889(6)	3267(5)	4137(4)	628(30)
O(4)	-4242(5)	3189(4)	4297(3)	825(22)
C(5)	-2658(6)	3849(5)	3560(3)	565(27)
C(6)	-2578(10)	4786(5)	3807(4)	766(33)
C(7)	-1388(9)	4926(6)	4310(4)	759(33)
C(8)	104(8)	4639(6)	4042(4)	770(34)
C(9)	-1190(8)	3693(5)	3268(4)	617(29)
O(9)	-955(6)	3693(4)	2695(3)	864(23)
C(10)	1346(9)	3287(6)	3442(4)	685(32)
O(10)	2417(6)	3696(4)	3412(3)	900(23)
C(11)	1271(9)	2363(6)	3157(5)	864(36)
C(12)	1611(13)	2420(8)	2417(4)	1174(48)
C(13)	2301(12)	1755(8)	3511(6)	1183(49)
C(14)	1236(10)	4701(6)	4595(4)	922(40)
C(15)	796(13)	4538(8)	5293(5)	1220(52)
C(16)	2080(17)	4534(10)	5727(6)	1513(67)
C(17)	2310(15)	4890(8)	6274(6)	1247(54)
C(18)	1323(25)	5469(13)	6599(8)	2161(106)
C(19)	3684(17)	4743(11)	6649(6)	1782(78)
C(20)	-3809(8)	3743(6)	3037(4)	776(34)
C(21)	-4063(9)	2799(8)	2825(5)	944(44)
C(22)	-3853(11)	2448(11)	2236(6)	1208(59)
C(23)	-3173(16)	2902(12)	1661(6)	1656(71)
C(24)	-4234(16)	1482(11)	2109(9)	1775(83)
C(25)	-1441(12)	5889(6)	4558(5)	1007(42)
C(26)	-2646(16)	6025(9)	5020(6)	1408(64)
C(27)	-3621(16)	6619(12)	5007(8)	1505(75)
C(28)	-3651(21)	7341(16)	4521(11)	2719(146)
C(29)	-4796(20)	6665(13)	5484(12)	2469(119)
C(30)	562(11)	5267(6)	3471(5)	988(40)

Table 2. *Bond Distances* [Å] *and Angles* [°]

C(1)–C(2)	1.518(10)	C(10)–O(10)	1.191(9)
C(1)–C(8)	1.589(11)	C(10)–C(11)	1.528(12)
C(1)–C(9)	1.523(10)	C(11)–C(12)	1.548(13)
C(1)–C(10)	1.553(10)	C(11)–C(13)	1.527(12)
C(2)–O(2)	1.247(8)	C(14)–C(15)	1.507(14)
C(2)–C(3)	1.423(10)	C(15)–C(16)	1.504(16)
C(3)–C(4)	1.353(10)	C(16)–C(17)	1.261(14)
C(4)–O(4)	1.327(8)	C(17)–C(18)	1.447(20)
C(4)–C(5)	1.493(10)	C(17)–C(19)	1.526(18)
C(5)–C(6)	1.517(11)	C(20)–C(21)	1.522(14)
C(5)–C(9)	1.529(10)	C(21)–C(22)	1.333(15)
C(5)–C(20)	1.535(10)	C(22)–C(23)	1.509(18)
C(6)–C(7)	1.540(11)	C(22)–C(24)	1.540(20)

Table 2 (cont.)

C(7)–C(8)	1.575(11)	C(25)–C(26)	1.495(15)
C(7)–C(25)	1.554(12)	C(26)–C(27)	1.293(17)
C(8)–C(14)	1.559(11)	C(27)–C(28)	1.484(24)
C(8)–C(30)	1.572(11)	C(27)–C(29)	1.479(21)
C(9)–O(9)	1.191(9)	O(4) · · · O(2')[a]	2.595(9)
		H(O4) · · · O(2')[a]	1.675(6)
C(2)–C(1)–C(8)	110.5(6)	C(14)–C(8)–C(30)	108.1(7)
C(2)–C(1)–C(9)	109.4(6)	C(5)–C(9)–O(9)	123.6(7)
C(2)–C(1)–C(10)	106.5(6)	C(1)–C(9)–O(9)	121.7(7)
C(8)–C(1)–C(9)	104.9(6)	C(1)–C(9)–C(5)	114.2(6)
C(8)–C(1)–C(10)	116.5(6)	C(1)–C(10)–O(10)	121.8(8)
C(9)–C(1)–C(10)	108.9(6)	C(1)–C(10)–C(11)	118.0(7)
C(1)–C(2)–O(2)	116.7(6)	O(10)–C(10)–C(11)	120.2(8)
C(1)–C(2)–C(3)	121.0(6)	C(10)–C(11)–C(12)	108.1(8)
O(2)–C(2)–C(3)	122.4(7)	C(10)–C(11)–C(13)	110.5(8)
C(2)–C(3)–C(4)	120.5(7)	C(12)–C(11)–C(13)	111.4(8)
C(3)–C(4)–C(5)	124.1(6)	C(8)–C(14)–C(15)	119.1(8)
C(3)–C(4)–O(4)	123.0(7)	C(14)–C(15)–C(16)	109.7(11)
C(5)–C(4)–O(4)	112.9(6)	C(15)–C(16)–C(17)	131.4(14)
C(4)–C(5)–C(6)	107.8(6)	C(16)–C(17)–C(18)	124.0(14)
C(4)–C(5)–C(9)	110.4(6)	C(16)–C(17)–C(19)	122.0(14)
C(4)–C(5)–C(20)	112.5(6)	C(18)–C(17)–C(19)	114.1(12)
C(6)–C(5)–C(9)	103.4(6)	C(5)–C(20)–C(21)	114.2(7)
C(6)–C(5)–C(20)	111.5(7)	C(20)–C(21)–C(22)	127.8(11)
C(9)–C(5)–C(20)	110.8(6)	C(21)–C(22)–C(23)	125.8(15)
C(5)–C(6)–C(7)	112.9(7)	C(21)–C(22)–C(24)	120.1(13)
C(6)–C(7)–C(8)	112.5(7)	C(23)–C(22)–C(24)	114.0(14)
C(6)–C(7)–C(25)	109.0(7)	C(7)–C(25)–C(26)	111.2(9)
C(8)–C(7)–C(25)	113.9(7)	C(25)–C(26)–C(27)	128.9(15)
C(1)–C(8)–C(7)	109.0(6)		
C(1)–C(8)–C(14)	110.8(7)	C(26)–C(27)–C(29)	123.7(19)
C(1)–C(8)–C(30)	109.1(6)	C(28)–C(27)–C(29)	113.0(16)
C(7)–C(8)–C(14)	110.3(7)	C(4)–O(4)–H(O4)	100.6(5)
C(7)–C(8)–C(30)	109.6(7)	O(4)–H(O4) · · · O(2')[a]	132.2(3)

[a]) Symmetry operation *i*: $-0.5+x, 0.5-y, 1-z$.

*4-Hydroxy-8-*exo*-methyl-5,7-*exo*-bis(3-methylbut-2-enyl)-1-(2-methyl-1-oxobutyl)-8-*endo*-(4-methylpent-3-enyl)bicyclo[3.3.1]non-3-ene-2,9-dione (= Hyperevolutin B; 2).* White powder. M.p. 132–137°. TLC (SiO_2, light petroleum ether/AcOEt/AcOH 70:30:3): R_f 0.27, orange with *Godin* reagent. UV same as for 1. ^{1}H-NMR (200 MHz, $CDCl_3$): 6.01 (*s*, H–C(3)); 5.18–4.84 (br. *m*, H–C(16), H–C(21), H–C(26)); 3.72 (*d*, *J* = 19), 3.46 (*d*, *J* = 19, CH_2(3) of the minor diketo tautomer); 2.64–2.39 (*m*, CH_2(20)); 2.11–1.75 (br. *m*); 1.70, 1.68, 1.65, 1.58, 1.57, (CH_3(18), CH_3(19), CH_3(23), CH_3(24), CH_3(28), CH_3(29)); 1.53–1.21 (br. *m*); 1.14 (*d*, *J* = 6.7, CH_3(12)); 1.04 (*s*, CH_3(30)); 0.83 (*dd*, *J* = 7.4, 7.4, CH_3(31)). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 210.1, 206.6 (C(9), C(10)); 185.7, 184.7 (C(2), C(4)); 135.7, 133.5, 131.4 (C(17), C(22), C(27)); 124.7, 122.4, 119.1 (C(16), C(21), C(26)); 110.0 (C(3)); 80.4, 59.1, 48.5 (C(1), C(5), C(8)); 48.7 (C(11)); 42.4 (C(7)); 39.0, 37.0, 29.3, 27.8, 27.6, 24.8 (C(6), C(13), C(14), C(15), C(20), C(25)); 26.0, 25.8, 25.7 (C(19), C(24), C(29)); 18.1, 18.0, 17.7 (C(18), C(23), (C(28)); 16.6 (C(12)); 14.2 (C(30)); 11.5 (C(31)). DCI-MS: 500 ($[M + NH_4]^+$), 483 ($[M + H]^+$).

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TI - ANTITUMOR ACTIVITY OF MEDICINAL PLANTS FROM THE LITHUANIAN SSR USSR 6. COMMON ST.-JOHN'S-WORT AND CHAMOMILLA-RECUTITA
AW - ** Miscellaneous Descriptors **
HYPERICUM-PERFORATUM RAT SARCOMA 45 CHOLANGIOMA PC-1
AU - VALAVICHYUS YU M; IVANAUSKAS V P; YASKONIS YU A
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AB - The effect of oil extracts of the St.-John's-wort Hypericum perforatum and Chamomilla recutita on the growth of sarcoma 45 and cholangioma PC-1 was studied in rats. The administration of the extracts inhibited the growth of tumors and increased the body weight of the animals. Data were presented on the effect of various doses of the extracts on the inhibition rate of the tumors.

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ANTITUMOR ACTIVITY OF HERBS OF THE LITHUANIAN SSR

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UNITED STATES PATENT AND TRADEMARK OFFICE
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ANTITUMOR ACTIVITY OF HERBS OF THE LITHUANIAN SSR
(6. Hypericum perforatum L. and Chamomilla recutita (L.) Rauschert)

[Protivoopukholeviya aktivnosti' lekarstvennykh rastenii Litovskoi SSR
(6. Zveroboi prodyryavlennyi i romashka obodrannya)]

1. Introduction. In the search for new antitumor agents among pharmaceutical plants, attention has been drawn to plants such as St. John's wort (*Hypericum perforatum L.*) and German chamomile (*Chamomilla recutita* (L.) Rauschert).

St. John's wort herb contains up to 12% tannin substances, 0.4% colorants, 0.59 glycosides, up to 55 mg% carotene, up to 0.24% essential oil, 3.57% organic acids, flavonoids, mineral substances and other substances [1].

According to [1-4], an oil extract from St. John's wort herb is used as a wound-healing agent. In addition, a complex polyphenol preparation has been obtained from this drug raw material – novoimanin, which is used topically in cases of infectious wounds, burns, trophic ulcers and gynecological diseases. The St. John's wort preparation, which contains catechols, has vitamin P activity. Preparations of St. John's wort are used in cases of migraine headache, vegetative dystonia, secondary anemia, nocturnal enuresis night-terrors, and also in cases of induration of the breasts and formation of tumors in various organs.

Flowers of German chamomile contain 0.2-0.54% essential oil, consisting of the main biologically active substance – hamazulen (40.1-82.4 mg%), its precursor – prohamazulen, and other monoterpenes and sesquiterpenes [5, 6].

German chamomile preparations have spasmolytic and anti-inflammatory activity, and stimulate processes of regeneration and healing of tissues in animals with experimental stomach ulcers [5].

The goal of this study was an investigation of the effect of preparations of St. John's wort and German chamomile on the growth of sarcoma 45 and cholangioma RS-1 in an experiment.

2. Technique. St. John's wort and German chamomile raw materials collected in 1982 were used in the experiments.

To prepare an oil extract of St. John's wort, 1 part ground St. John's wort was covered with 4 parts by weight fresh sunflower seed oil. The resulting mixture was heated at a maximum temperature of 60°C and held in a heated state until the St. John's wort raw material became brittle, but the oil had not yet attained a brownish-red color. The resulting extract was left standing for 1 day, filtered through three layers of gauze and poured into orange glass 100-mL bottles.

To prepare the chamomile extract, 1 part pharmacopoeic chamomile raw material was mixed with 20 parts Freon 12 and extracted in a closed system for 3 h, then strained, the Freon 12 was evaporated out, as a result of which a thick blue-green extract was obtained.

Before the beginning of the experiment, one part of the chamomile extract was mixed with 20 parts fresh sunflower seed oil, producing an oil extract of chamomile.

The experiments were carried out on nonlinear white female rats weighing 110-140 g grafted with sarcoma 45 (56 rats) and cholangioma RS-1 (56 rats).

The oil extract of St. John's wort was tested on rats in doses of 0.25, 0.50, 1.0 and 2.0 mg/kg, while the oil extract of chamomile was tested in doses of 0.5 and 1.0 mg/kg. The tested preparations were administered to the rats daily for 10 days intraperitoneally once a day.

The antitumor effect of the preparations was evaluated from the degree of inhibition of tumor growth.

The results were statistically analyzed [7].

Results and discussion

The investigation of the antitumor effect of the oil extract of St. John's wort with respect to sarcoma 45 showed (Table 1, a) that the inhibition of tumor growth was 18% for 10 administrations of the preparation in a dose of 0.25 mg/kg, while the weight of the spleen and the body weight increased respectively by 21.4 and 15% over the control. Administration of the preparation in a dose of 0.5 mg/kg produced inhibition of tumor growth by 37%, while the weight of the spleen and the body weight increased respectively by 5.0 and 10.4%.

Table 1. Effect of oil extract of St. John's wort on the growth of sarcoma 45 and cholangioma RS-1.

(1) Группа крыс	(2) Доза, мг/кг	(3) Торможение роста опухоли		(4) Изменение массы (5) селезенки		(4) Изменение массы (6) тела	
		%	P<	%	P<	%	P<
			(7) а. Саркома 45				
I (контроль) (9)	–	–	–	–	–	+6,5	0,1
II	0,25	18	0,5	+21,4	0,2	+15,0	0,005
III (контроль) (9)	–	–	–	–	–	+7,0	0,2
IV	0,5	37	0,01	+5,0	0,5	+10,4	0,025
V (контроль) (9)	–	–	–	–	–	–0,9	0,5
VI	1,0	42	0,05	+30	0,01	+5,7	0,1
VII	2	31	0,025	+20	0,025	+2,4	0,1
			(8) б. Холангиома РС-1				
I (контроль) (9)	–	–	–	–	–	–4,1	0,4
II	1	36	0,025	–25	0,1	+7,2	0,2

Key:
1 Group of rats
2 Dose, mg/kg
3 Inhibition of tumor growth
4 Change of weight
5 Spleen
6 Body
7 a. Sarcoma 45
8 b. Cholangioma RS-1
9 (Control)

For ten administrations of the preparation in a dose of 1.0 mg/kg, the inhibition of tumor growth was 42%, while the weight of the spleen and body weight increased respectively by 30 and 5.7%. When the preparation was administered in a dose of 2 mg/kg, the inhibition of tumor growth was 31%, while the weight of the spleen and body weight increased respectively by 20 and 2.4%.

Growth of cholangioma RS-1 (Table 1, b) was inhibited by the oil extract of St. John's wort in a dose of 1 mg/kg by 36%, while the weight of the spleen decreased by 25% and the body weight increased by 7.2% compared to the control.

The study of the effect of the oil extract of St. John's wort on the lifespan of the rats showed that the administration of 1 mg/kg preparation increased the 100% survivability of the rats by 54%, and the average lifespan by 71%. In 1 experimental rat, the tumor was completely resorbed.

The investigation of the antitumor effect of the oil extract of chamomile with respect to sarcoma 45 (Table 2, a) showed that ten administrations of the preparation in a dose of 0.5 mg/kg produced an inhibition of tumor growth by 20%, while the weight of the spleen decreased by 23.3%, and the body weight increased by 4.2%.

Increasing the dose of the preparation to 1 mg/kg inhibited tumor growth by 17%, while the weight of the spleen decreased by 5.6%, and the body weight by 0.7%.

The growth of cholangioma RS-1 (Table 2, b) was inhibited by 35% by the tested preparation in the dose of 0.5 mg/kg, while the weight of the spleen and body weight increased respectively by 3.7 and 8.3%. Increasing the dose of the preparation to 1 mg/kg inhibited tumor growth by 51% and increased the weight of the spleen by 12.5% and the body weight by 6.4%, compared to the control.

Table 2. Effect of oil extract of German chamomile on the growth of sarcoma 45 and cholangioma RS-1.

(1) Группа крыс	(2) Доза, мг/кг	(3) Торможение роста опухоли		(4) Изменение массы (5) селезенки		(6) тела	
		%	P<	%	P<	%	P<
				(7) а. Саркома 45			
I (контроль) (9)	–	–	–	–	–	+3,8	0,4
II	0,5	20	0,5	–23,3	0,05	+4,2	0,4
III	1,0	17	0,5	–5,6	0,5	–0,7	0,5
				(8) б. Холангиома РС-1			
I (контроль) (9)	–	–	–	–	–	–4,1	0,4
II	0,5	35	0,5	+3,7	0,05	+8,3	0,2
III	1,0	51	0,4	+12,5	0,4	+6,4	0,4

Key:
1 Group of rats
2 Dose, mg/kg
3 Inhibition of tumor growth
4 Change of weight
5 Spleen
6 Body
7 a. Sarcoma 45
8 b. Cholangioma RS-1
9 (Control)

Based on the above, the oil extract of St. John's wort and the oil extract of German chamomile are capable of reliably inhibiting the growth of grafted sarcoma 45 and cholangioma RS-1 and increasing the body weight of the experimental animals.

4. Findings

1. Experiments conducted on nonlinear white female rats weighing 110-140 g with grafted sarcoma 45 (56 rats) and cholangioma RS-1 (56 rats) showed that an oil extract of St. John's wort and an oil extract of German chamomile (raw material from harvest of 1982) had the ability to inhibit the growth of tumors and to increase the body weight of the experimental animals.

2. The reliable maximum inhibition of tumor growth was achieved both in the rats with sarcoma 45 and in the rats with cholangioma RS-1 when the oil extract of St. John's wort was administered in a dose of 1 mg/kg (42 and 36%, respectively).

3. Administering the oil preparation of chamomile 10 times in doses of 0.5 and 1.0 mg/kg to rats with sarcoma 45 inhibited the tumor growth respectively by 20 and 17%. 10 administrations of the oil preparation of chamomile in doses of 0.5 and 1.0 mg/kg to rats with grafted cholangioma RS-1 inhibited tumor growth respectively by 35 and 51%, and had a favorable effect on the weight of the spleen and the body weight of the rats.

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УДК 615.31/36-006

Противоопухолевая активность лекарственных растений Литовской ССР (6. Зверобой продырявленный и ромашка ободранная)

Балавичюс Ю. М., Иванаускас В. П., Ясконис Ю. А.

1. Введение. В поисках новых противоопухолевых средств среди лекарственных растений внимание привлекли такие растения, как зверобой продырявленный (Hypericum perforatum L.) и ромашка ободранная (Chamomilla recutita (L.) Rauschert).

Трава зверобоя продырявленного содержит до 12% дубильных веществ, 0,4% красящих веществ, 0,5% гликозидов, до 55 мг% каротина, до 0,24% эфирного масла, 3,57% органических кислот, флавоноидов, минеральных и других веществ [1].

По данным [1—4], масляное извлечение из травы зверобоя продырявленного используют как ранозаживляющее средство. Кроме того, из этого лекарственного сырья получен комплексный полифенольный препарат — новоиманин, применяемый наружно при инфекционных ранах, ожогах, трофических язвах и гинекологических заболеваниях. Препарат зверобоя продырявленного, содержащий катехины, обладает Р-витаминной активностью. Препараты зверобоя продырявленного применяются при мигрени, вегетативной дистонии, вторичной анемии, Enuresis nocturna, Pavor nocturnus, а также при затвердении молочной железы и образовании опухолей в различных органах.

Соцветия ромашки ободранной содержат 0,2—0,54% эфирного масла, состоящего из основного биологически активного вещества — хамазулена (40,1—82,4 мг%), его предшественника — прохамазулена и других монотерпенов и сесквитерпенов [5, 6].

Препараты ромашки ободранной оказывают спазмолитическое и противовоспалительное действие, а у животных с экспериментальными язвами желудка стимулируют процессы регенерации и заживления тканей [5].

Целью настоящей работы явилось изучение влияния препаратов зверобоя продырявленного и ромашки ободранной на рост саркомы 45 и холангиомы РС-1 в эксперименте.

2. Методика. В опытах использовали сырье зверобоя продырявленного и ромашки ободранной, собранное в 1982 г.

Для приготовления масляного экстракта зверобоя 1 часть измельченного сырья зверобоя заливали 4 весовыми частями свежего подсолнечного масла. Полученную смесь подогревали при температуре не выше 60 °С и выдерживали в подогретом состоянии до тех пор, пока сырье зверобоя не становилось хрупким, а масло не приобретало буро-красный цвет. Полученный экстракт отстаивали в течение 1 сут, фильтровали через 3-слойную марлю и разливали во флаконы из оранжевого стекла емкостью 100 мл.

Для приготовления экстракта ромашки 1 часть фармакопейного сырья ромашки заливали 20 частями фреона 12 и экстрагировали в закрытой системе в течение 3 ч, затем процеживали, испаряли фреон 12, в результате чего получался густой сине-зеленый экстракт.

Перед началом опыта 1 часть экстракта ромашки смешивали с 20 частями свежего подсолнечного масла, в результате чего получался масляный препарат ромашки.

Опыты проводили на нелинейных белых крысах-самках массой по 110—140 г с привитыми саркомой 45 (56 крыс) и холангиомой РС-1 (56 крыс).

Масляный экстракт зверобоя тестировали на крысах в дозах 0,25, 0,50, 1,0 и 2,0, а масляный препарат ромашки — в дозах 0,5 и 1,0 мг/кг. Исследуемые препараты вводили крысам ежедневно в течение 10 сут внутрибрюшинно 1 раз в сутки.

Противоопухолевый эффект препаратов оценивали по степени торможения роста опухоли.

Полученные результаты обработаны статистически [7].

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3. Результаты и их обсуждение

Исследование противоопухолевого эффекта масляного экстракта зверобоя в отношении саркомы 45 показало (табл. 1а), что при 10-кратном введении крысам препарата в дозе 0,25 мг/кг торможение роста опухоли составляло 18 %, а масса селезенки и тела увеличилась соответственно на 21,4 и 15% по сравнению с контролем. Введение препарата в дозе 0,5 мг/кг вызывало торможение роста опухоли на 37%, а массу селезенки и тела увеличивало соответственно на 5,0 и 10,4%.

Таблица 1. **Влияние масляного экстракта зверобоя продырявленного на рост саркомы 45 и холангиомы РС-1**

Группа крыс	Доза, мг/кг	Торможение роста опухоли		Изменение массы			
				селезенки		тела	
		%	$P<$	%	$P<$	%	$P<$
а. Саркома 45							
I (котроль)	—	—	—	—	—	+6,5	0,1
II	0,25	18	0,5	+21,4	0,2	+15,0	0,005
III (контроль)	—	—	—	—	—	+7,0	0,2
IV	0,5	37	0,01	+5,0	0,5	+10,4	0,025
V (контроль)	—	—	—	—	—	−0,9	0,5
VI	1,0	42	0,05	+30	0,01	+5,7	0,1
VII	2	31	0,025	+20	0,025	+2,4	0,1
б. Холангиома РС-1							
I (контроль)	—	—	—	—	—	−4,1	0,4
II	1	36	0,025	−25	0,1	+7,2	0,2

При 10-кратном введении препарата в дозе 1,0 мг/кг торможение роста опухоли составляло 42%, а масса селезенки и тела увеличилась соответственно на 30 и 5,7%. При введении препарата в дозе 2 мг/кг торможение роста опухоли составляло 31%, а масса селезенки и тела увеличивалась соответственно на 20 и 2,4%.

Рост холангиомы РС-1 (табл. 1б) масляный экстракт зверобоя в дозе 1 мг/кг тормозил на 36%, массу селезенки уменьшал на 25, а массу тела увеличивал на 7,2% по сравнению с контролем.

Исследование действия масляного экстракта зверобоя на продолжительность жизни крыс показало, что введение 1 мг/кг препарата повышало 100% выживаемость крыс на 54, а среднюю продолжительность жизни — на 71%. У 1 подопытной крысы опухоль полностью рассосалась.

Исследование противоопухолевого эффекта масляного препарата ромашки в отношении саркомы 45 (табл. 2а) показало, что при 10-кратном введении крысам препарата в дозе 0,5 мг/кг торможение роста опухоли составляло 20%, масса селезенки при этом уменьшалась на 23,3, а масса тела увеличивалась на 4,2%.

Увеличение дозы препарата до 1 мг/кг тормозило рост опухоли на 17%, массу селезенки уменьшало на 5,6, а массу тела — на 0,7%.

Рост холангиомы РС-1 (табл. 2б) испытуемый препарат в дозе 0,5 мг/кг тормозил на 35%, а массу селезенки и тела увеличивал соот-

и холангиомы РС-1 ромашки ободранной на рост саркомы 45

Группа крыс	Доза, мг/кг	Торможение роста опухоли		Изменение массы			
				селезенки		тела	
		%	$P<$	%	$P<$	%	$P<$
		а. Саркома 45					
I (контроль)	—	—	—	—	—	—	—
II	0,5	20	0,5	−23,3	0,05	+3,8	0,4
III	1,0	17	0,5	−5,6	0,5	+4,2	0,4
						−0,7	0,5
		б. Холангиома РС-1					
I (контроль)	—	—	—	—	—	—	—
II	0,5	35	0,5	+3,7	0,05	−4,1	0,4
III	1,0	51	0,4	+12,5	0,4	+8,3	0,2
						+6,4	0,4

ветственно на 3,7 и 8,3%. Увеличение дозы препарата до 1 мг/кг тормозило рост опухоли на 51% и увеличивало массу селезенки на 12,5, а массу тела — на 6,4% по сравнению с контролем.

Исходя из вышеизложенного, масляный экстракт зверобоя продырявленного и масляный препарат ромашки ободранной способны достоверно тормозить рост перевиваемой саркомы 45 и холангиомы РС-1 и увеличивать массу тела подопытных животных.

4. Выводы

1. Опыты, проведенные на нелинейных белых крысах-самках массой по 110—140 г с привитыми саркомой 45 (56 крыс) и холангиомой РС-1 (56 крыс), показали, что масляный экстракт зверобоя продырявленного и масляный препарат ромашки ободранной (сырье урожая 1982 г.) обладали способностью тормозить рост опухолей и увеличивать массу тела подопытных животных.

2. Достоверное максимальное торможение роста опухоли как у крыс с саркомой 45, так и у крыс с холангиомой РС-1 было достигнуто при введении масляного экстракта зверобоя в дозе 1 мг/кг (соответственно 42 и 36%).

3. 10-кратное введение масляного препарата ромашки в дозах 0,5 и 1,0 мг/кг крысам с саркомой 45 тормозило рост опухоли соответственно на 20 и 17%. 10-кратное введение масляного препарата ромашки в дозах 0,5 и 1,0 мг/кг крысам с привитой холангиомой РС-1 тормозило рост опухоли соответственно на 35 и 51% и положительно влияло на массу селезенки и тела крыс.

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kamai 35 ir

Lietuvos TS
Biochemijos
Botanikos ir

УДК 615.31

Противоопу
продырявле
конис Ю. А

Опыты,
с привитым
масляный э
ляный прег
урожая 198
тела подопы
Достове
так и у кр
та зверобоя
10-крат
мой 45 тор
препарата
35 и 51% и
Таблиц

Valavičius J

Anti-tumor
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8. C sertic

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Lietuvos TSR vaistinių augalų priešnavikinis aktyvumas
(6. Paprastoji jonažolė ir vaistinė ramunė)

Valavičius J., Ivanauskas V., Jaskonis J.

Reziumė

Paprastosios jonažolės (Hypericum perforatum L.) ir vaistinės ramunės (Chamomilla recutita (L.) Rauschert) žaliavos (1982 m. derliaus) preparatų priešnavikiniam aktyvumui tirti panaudota 112 baltųjų nelinijinių žiurkių patelių, kurioms buvo įskiepyta sarkoma 45 (56 žiurkėms) ir RS-1 (56 žiurkėms).

Nustatyta, kad jonažolės saulėgrąžų aliejaus ekstraktas ir ramunės aliejinis preparatas stabdo auglių augimą ir padidina bandomųjų žiurkių bendrą svorį, palyginti su kontrolinėmis.

10 dienų po 0,5, 1,0 ir 2 mg/kg leistas į peritoneumą jonažolės saulėgrąžų aliejaus ekstraktas sarkomos 45 augimą stabdė atitinkamai 37, 42 ir 31%.

10 dienų po 0,5 ir 1,0 mg/kg leistas ramunės saulėgrąžų aliejaus preparatas sarkomos 45 augimą stabdė atitinkamai 17 ir 20%, o cholangiomos RS-1 augimą — atitinkamai 35 ir 51% ir padidino žiurkių bendrą bei blužnies svorį, palyginti su kontrolinėmis.

Lietuvos TSR Mokslų Akademijos
Biochemijos institutas
Botanikos institutas

Gauta
1985.III.4

УДК 615.31/36-006

Реферат

Противоопухолевая активность лекарственных растений Литовской ССР (6. Зверобой продырявленный и ромашка ободранная). Валавичюс Ю. М., Иванаускас В. П., Ясконис Ю. А. — Тр. АН ЛитССР. Сер. В, 1986, т. 3(95), с. 110—113.

Опыты, проведенные на нелинейных белых крысах-самках массой по 110—140 г с привитыми саркомой 45 (56 крыс) и холангиомой РС-1 (56 крыс), показали, что масляный экстракт сырья зверобоя продырявленного (Hypericum perforatum L.) и масляный препарат ромашки ободранной (Chamomilla recutita (L.) Rauschert) (сырье урожая 1982 г.) обладали способностью тормозить рост опухолей и увеличивать массу тела подопытных животных.

Достоверное максимальное торможение роста опухоли как у крыс с саркомой 45, так и у крыс с холангиомой РС-1 было достигнуто при введении масляного экстракта зверобоя в дозе 1 мг/кг (соответственно 42 и 36%).

10-кратное введение препарата ромашки в дозах 0,5 и 1 мг/кг крысам с саркомой 45 тормозило рост опухоли соответственно на 20 и 17%, а введение этих доз препарата крысам с холангиомой РС-1 тормозило рост опухоли соответственно на 35 и 51% и положительно влияло на массу селезенки и тела крыс.

Таблиц 2, библиографий 7.

Valavičius J., Ivanauskas V., Jaskonis J.

Anti-tumor activity of herbs of the Lithuanian SSR
(6. Hypericum perforatum L. and Chamomilla recutita (L.) Rauschert)

XP-002139269

AN - PREV198783099063
TI - ANTITUMOR ACTIVITY OF MEDICINAL PLANTS FROM THE LITHUANIAN SSR USSR 6. COMMON ST.-JOHN'S-WORT AND CHAMOMILLA-RECUTITA
AW - ** Miscellaneous Descriptors **
HYPERICUM-PERFORATUM RAT SARCOMA 45 CHOLANGIOMA PC-1
AU - VALAVICHYUS YU M; IVANAUSKAS V P; YASKONIS YU A
AUAF- INST. BIOCHEM., ACAD. SCI. LITH. SSR, VILNIUS, USSR.
PUB - Lietuvos TSR Mokslu Akademijos Darbai Serija C Biologijos Mokslai
- 1986
NR - 3
PG - 110-113
PD - 1986-00-00
DT - Article
LA - RS
IRN - ISSN 0131-3851

P.D. 1986
p. = 1

AB - The effect of oil extracts of the St.-John's-wort Hypericum perforatum and Chamomilla recutita on the growth of sarcoma 45 and cholangioma PC-1 was studied in rats. The administration of the extracts inhibited the growth of tumors and increased the body weight of the animals. Data were presented on the effect of various doses of the extracts on the inhibition rate of the tumors.
PBC - 24008 25840 26135 86375
PCC - 10066 22002-24004-24008*51522 54000-

The Hypericum Home Page

Scientific Description of *Hypericum Perforatum*

Edited version of the ESCOP (European Scientific Corporation of Phototherapy) Proposal of Product Characteristics

Definition

The drug St. John's wort consists of the dried above-ground part *of Hypericum perforatum L* collected shortly before or during the flowering period. It contains not less than 0.04% naphthodianthrones of the hypericin group (so-called total hypericin). Lower parts of the stem contain few active ingredients (2,3,4).

Components (4-21)

- Hypericum extracts contain at least ten components or groups of components that may contribute to the pharmacological effects. It is not yet possible to correlate the antidepressive mode of action with specific components; therefore, the pharmaceutical quality of the extracts was characterized on the basis of typical leading substances, especially the hypericins. The substances most involved in the antidepressant action are thought to be the hypericins and the flavonoids.
- The red-colored hypericins have been found in very few other plants while most of hypericum's other ingredients are common in the plant kingdom.
- The hypericins also have a photodynamic effect; sometimes they do not occur until the crude drug has been processed and exposed to light. The amount of total hypericin should be measured after light exposition, which transforms the biological precursors, protohypericin and protopseudo-hypericin, into hypericin and pseudohypericin (5-8).
- The concentration of hypericins (mainly hypericin and pseudohypericin) in buds and flowers can vary between 0.06% and 0.75%. The usual concentration is 0.1-0.15%, but lower concentrations (less than 0.1%) might result from harvesting of lower parts of the herb (4). A minimum content of 0.04% total hypericin is required for commercial use.

- Other possible active ingredients are polymerization products of hypericin, the flavonoids quercetin, hyperoside, quercitrin, isoquercitrin, rutin, campherol, luteolin, and 13-118-biapigenin, the total concentration of which can amount to 2% to 4%.
- The 1,3,6,7-tetra-hydroxyxanthone, the aglycone of the mangiferin found in other species of *Hypericum,* is only present in concentrations of 0.0004%.
- The procyanidines, which are related to the flavonoids, account for about 8%.
- Hyperforin, with a structure related to the hop bitters humulon and lupulon, contributes to about 2.8%.

Pharmaceutical form

Hypericum is available in tablets, capsules, drops and teas. It is also available as an oil for external use.

The oil cannot be recommended for internal use as an antidepressant.

Therapeutic indications

The official German commission monograph lists mild to moderate depressive states (22-51), fear, and nervous disturbances, and somatoform disturbances as clinical indications for hypericum.

Most of the scientific documentation on hypericum has been performed on mild to moderate depressions. Treatment of severe depressions (with suicidal, psychotic or severe melancholic features) with hypericum preparations is not yet recommended.

Clinical effect (22-51,109-110)

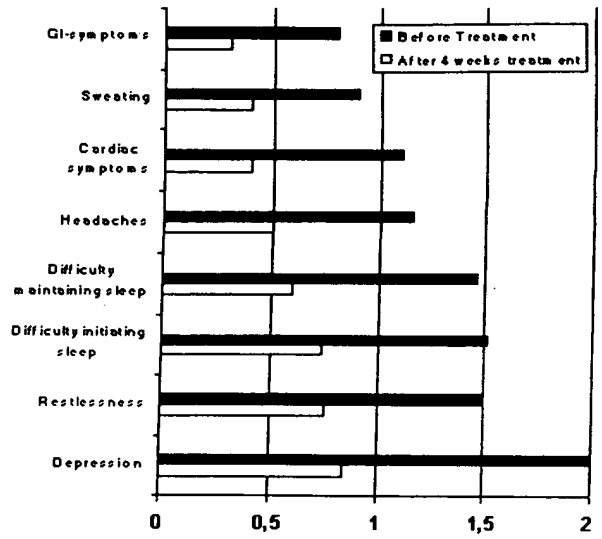
Besides numerous case reports and drug monitoring studies (with more than 5,000 patients) on the efficacy and safety of standardized St. John's wort preparations, 25 controlled double-blind studies (with more than 2,000 patients) have been conducted. The major indication was mild to moderate depressive disorders.

Sixteen of the studies compared hypericum with placebo (sugar-pills) and 9 with reference treatments (Imipramine-2 (34, 44), Amitryptilin-2 (32, 48), Maprotiline-1 (46), Desipramine-1(30), Diazepam-2 (27,29), and Light-therapy (47).

In most of the studies, both depressive symptoms (depressed

mood, anxiety, loss of interest, feelings of worthlessness, decreased activity) and secondary symptoms (sleep disturbance, lack of concentration, somatic complaints) improved significantly (see Figure 1) (25).

Fig. 1. Severity of symptoms before and after treatment with Hypericum for 4 weeks in 3250 patients measured by GCI



Results are summarized in Table 1. Some facts:

- The response rate has generally been between 50 and 80%, comparable to that of low- to medium dose treatment with "classic" synthetic antidepressants.
- In three of the trials (39,45,109) there was -- were no statistically significant difference between hypericum and placebo. In a criteria-based clinical review by Ernst (110) two of these studies also were judged as questionable because of methodological weaknesses. The third one is a yet unpublished study. They were all made with low dose hypericum test medications.
- Hypericum leads to an increase in deep sleep and does not impair cognitive functions or the ability to work or drive a car (83, 85).
- Hypericum has been shown to have a long-term effect on anxiety comparable to Bromazepam and Diazepam (29, 35).
- A Russian study (102) showed good results combining hypericum with psychotherapy to treat alcoholics with peptic ulcers.
- A preliminary study by Martinez et al. also showed an effect comparable to light therapy in the treatment of seasonal affective disorder (47).
- Anti-inflammatory and antibacterial effects of externally applied St. John's wort preparations have been reported and attributed to the presence of hyperforin. (1).

[General]

Dermatitis (eczema): Superficial skin inflammation, characterized histologically by epidermal edema and clinically by vesicles (when acute), poorly marginated redness, edema, oozing, crusting, scaling, usually pruritus, and lichenification caused by scratching or rubbing.

Authorities generally disagree about how to use the synonymous terms eczema and dermatitis. Often, eczema refers to vesicular dermatitis, but some authorities restrict eczema to mean chronic dermatitis. Some also refer to dermatitis as spongiotic dermatitis because spongiosis (intraepidermal edema) is a histologic feature.

Contact Dermatitis

Acute or chronic inflammation, often asymmetric or oddly shaped, produced by substances contacting the skin and causing toxic (irritant) or allergic reactions.

Etiology and Pathogenesis

Contact dermatitis may be caused by a primary chemical irritant or by an allergen (ie, a type IV delayed hypersensitivity reaction--see Ch. 148).

Primary irritants may damage normal skin or irritate existing dermatitis. Clinically recognizable changes may occur within minutes of exposure to strong irritants (eg, acids, alkalis, phenol) or may take up to several days' exposure to weak or marginal irritants (eg, soap, detergents, acetone, or even water). The mechanisms by which these irritants damage the skin are different for different agents. For example, detergents activate keratinocytes, causing them to release inflammatory cytokines.

Allergic contact dermatitis patients may become allergic to substances that they have sometimes used for years or to drugs used to treat skin diseases. Allergens are captured by Langerhans' cells (a minor subpopulation of epidermal cells), which present them to T cells. Cytokines released from keratinocytes and Langerhans' cells may also contribute to sensitivity induction. It takes between 6 and 10 days (in the case of strong sensitizers, eg, poison ivy) to years (for weaker sensitizers) for patients to become sensitized. On reexposure to the sensitizer, patients may develop pruritus and dermatitis within 4 to 12 h.

Ingredients in topical drugs constitute a major cause of allergic contact dermatitis (see Table 111-1). Other commonly implicated substances include plants (eg, poison ivy), sensitizers used in the manufacture of shoes and clothing, metal compounds, dyes, and cosmetics. Many industrial agents can produce occupational dermatoses. Sensitivity to rubber accelerators or latex in gloves is a particular problem for many

health professionals. Sensitivity to latex condoms may preclude their use in some men.

Photoallergic and phototoxic contact dermatitides require exposure to light after topical application of certain chemicals. The chemicals (phototoxins) produce an exaggerated response to sunlight by acting as photosensitizers (see polymorphous light eruptions under Photosensitivity in Ch. 119). Aftershave lotions, sunscreens, and topical sulfonamides are commonly responsible for photoallergic contact dermatitis. Phototoxic contact dermatitis is commonly caused by certain perfumes, coal tar, psoralens, and oils used in manufacturing. Photoallergic and phototoxic contact dermatitides must be differentiated from photosensitivity reactions to systemic drugs.

Symptoms, Signs, and Course

Contact dermatitis ranges from transient redness to severe swelling with bullae (see Plate 111-1); pruritus and vesiculation are common. Any skin surface exposed to an irritant or sensitizing substance (including airborne ones) may be involved. Typically, the dermatitis is limited to the site of contact but may later spread.

The course varies. If the causative agent is removed, erythema disappears within a few days to weeks and blisters dry up. Vesicles and bullae may rupture, ooze, and crust. As inflammation subsides, scaling and some temporary thickening of the skin occur. Continued exposure to the causative agent or complications (eg, irritation from or allergy to a topical drug, excoriation, infection) may perpetuate the dermatitis.

Diagnosis

Contact dermatitis may resemble other types of dermatitis. Typical skin changes and a history of exposure facilitate diagnosis, but confirmation may require exhaustive questioning and extensive patch testing. The patient's occupation, hobbies, household duties, vacations, clothing, topical drug use, cosmetics, and spouse's activities must be considered. Knowing the characteristics of irritants or topical allergens and the typical distribution of lesions is helpful. The site of the initial lesion is often an important clue.

Patch testing (see Disorders with Type IV Hypersensitivity Reactions in Ch. 148) with standard contact allergens may be helpful. Test concentrations are important and, particularly for industrial agents or cosmetics, an industrial specialist should be consulted. Because patch testing may worsen an eruption in a very sensitive patient and may yield ambiguous results during acute dermatitis, it is often performed after the eruption subsides. However, a positive patch test does not necessarily identify the causative agent. For definitive diagnosis, a history of exposure to the test agent must be present in the areas where the

dermatitis originally occurred. Moreover, no reactions will be seen if the causative agent was not included in the tested chemicals.

Treatment

Unless the causative agent is identified and removed, treatment may be ineffective. Patients with photoallergic or phototoxic contact dermatitis should also avoid the photosensitizing chemical or exposure to light. In acute dermatitis, gauze or thin cloths dipped in water and applied to the lesions (30 min 4 to 6 times/day) are soothing and cooling. Blisters may be drained three times daily, but the tops should not be removed. An oral corticosteroid (eg, prednisone 60 mg/day) may be given (if not contraindicated) for 7 to 14 days in extensive cases, or even in limited cases when facial inflammation is severe. The prednisone dose can be decreased by 10 to 20 mg q 3 to 4 days. Topical corticosteroids are not helpful in the blistering phase, but once the dermatitis is less acute, a topical corticosteroid cream or ointment (see Ch. 110) can be rubbed in gently three times daily. Antihistamines are ineffective in suppressing allergic contact dermatitis but may blunt the itching.

Atopic Dermatitis

Chronic, pruritic, superficial inflammation of the skin, frequently associated with a personal or family history of allergic disorders (eg, hay fever, asthma).

Etiology

Susceptibility is genetic, but the disorder is triggered by various environmental agents and factors. Numerous inhalants and foods produce wheal and flare reactions on scratch or intradermal tests, but these reactions are usually not relevant; elimination does not usually cause remission, except sometimes in young patients. Patients with atopic dermatitis usually have high serum levels of reaginic (IgE) antibodies, peripheral eosinophilia, and high levels of cAMP phosphodiesterase in their WBCs, but the etiologic significance of these findings is unknown.

Symptoms, Signs, and Course

Atopic dermatitis may begin in the first few months of life, with red, weeping, crusted lesions on the face, scalp, diaper area, and extremities. In older children or adults, it may be more localized and chronic, typically appearing as erythema and lichenification in the antecubital and popliteal fossae and on the eyelids, neck, and wrists. The course is unpredictable. Although the dermatitis often improves by age 3 or 4 yr, exacerbations are common during childhood, adolescence, or adulthood.

Pruritus is constant; subsequent scratching and rubbing lead to an itch-scratch-rash-itch cycle. The dermatitis may become generalized (see below). Secondary bacterial infections and regional lymphadenitis are common. Frequent use of proprietary or prescribed drugs exposes the patient to many topical allergens, and contact dermatitis may aggravate and complicate atopic dermatitis, as may the generally dry skin that is common in these patients. Intolerance to primary environmental irritants is common, and emotional stress, ambient temperature or humidity

changes, bacterial skin infections, fragrances, fabric softeners, and wool garments commonly cause exacerbations.

Complications

Patients with long-standing atopic dermatitis may develop cataracts while in their 20s or 30s. Cataracts may be a feature of atopy or may result from extensive systemic and topical corticosteroid use. Herpes simplex may induce a generalized painful vesicular eruption and sometimes a grave febrile illness (eczema herpeticum) in atopic patients.

House dust mites in bedding, upholstered furniture, and carpeting may significantly exacerbate atopic dermatitis.

Diagnosis

Diagnosis is based on the distribution and duration of lesions and often on a family history of atopic disorders and the presence of lichenification. Because atopic dermatitis is often hard to differentiate from seborrheic dermatitis in infants or from contact dermatitis at any age, the physician should examine the patient several times before making a definitive diagnosis. The physician must be careful not to attribute all subsequent skin problems to an atopic diathesis.

Treatment

Precipitating agents and complex topical drugs should be avoided if possible. Corticosteroid creams or ointments applied three times daily are most effective. Because topical corticosteroids are expensive, supplemental use of white petrolatum, hydrogenated vegetable oil (as for cooking), or hydrophilic petrolatum (unless the patient is allergic to lanolin) may be advisable. These emollients, applied between corticosteroid applications, also help hydrate the skin, which is important. Prolonged, widespread use of high-potency corticosteroid creams or ointments should be avoided in infants because adrenal suppression may ensue.

Older adults may benefit from treatment with ultraviolet radiation B, psoralen plus high-intensity ultraviolet A (PUVA--see under Psoriasis in Ch. 117), or narrow band ultraviolet A without psoralen. Because of its potential long-term side effects, however, PUVA is rarely indicated for children or young adults.

Bathing should be minimized if it exacerbates symptoms; soap should not be used on dermatitic areas because it may be drying and irritating. Oils help lubricate the skin, and corticosteroid or emollient ointments should be applied within 3 min of bathing, before the skin is dried, to enhance effectiveness.

Antihistamines may provide some relief but are often sedating and anticholinergic. Doxepin, a dibenzoxepin tricyclic compound, is a very active antihistamine that also has a useful psychotherapeutic effect in pruritic patients. The starting dose is 25 to 50 mg po at bedtime. Doxepin cream 5% may be applied qid, but percutaneous absorption may cause systemic symptoms. Hydroxyzine hydrochloride 25 mg tid or qid (for children, 2 mg/kg/day in divided doses q 6 h)may also be useful. Diphenhydramine 25 to 50 mg may be given at bedtime, when pruritus is usually worst.

Fingernails should be kept short to minimize excoriations and secondary infections. For secondary infections, an oral penicillinase-resistant penicillin or a cephalosporin qid is advised.

Oral corticosteroids should be considered a last resort but, if given, are best used in 1- to 2-wk courses. Stunted growth, osteoporosis, and other side effects occur with prolonged use of systemic corticosteroids, and rebound exacerbations on stopping therapy are frequent. Alternate-day use of corticosteroids (eg, for adults, prednisone 20 to 40 mg every other morning) may help reduce side effects. The initial dose should be continued for several weeks, then slowly decreased while the patient starts using topical drugs.

For unusually widespread, recalcitrant, or disabling cases, experimental treatments, such as oral emulsified cyclosporine 1.5 to 2.5 mg/kg bid in adults, have proven useful. Tacrolimus is a topical immunosuppressive ointment without systemic effects. It may be useful in children and adults with severe atopic dermatitis. Newly developed phosphodiesterase-4 inhibitors may become important therapy.

If atopic dermatitis resists home treatment, hospitalization, which provides closer psychologic and dermatologic attention and a change in environment, often accelerates improvement.

Chronic Dermatitis Of The Hands And Feet

The hands and feet are frequent sites of inflammatory eruptions--the hands because they are subjected to mechanical and chemical trauma; the feet because of the warm, moist conditions in shoes. Eruptions commonly become chronic and can be crippling at home or at work.

Contact dermatitis (see above) is common. Many allergens or irritants--caustics, strong soaps, detergents, organic solvents, vacuum cleaner dust, topical drugs--may cause or perpetuate the dermatitis. Occasionally, contact dermatitis that appears urticarial occurs in 10 to 20 min as a reaction to fresh foods. In any dermatitis of the feet, every effort should be made to obtain patch-test evidence of sensitivity to a component of shoes because sensitivity limits the choice of footwear.

"Housewives' eczema," which affects persons who frequently immerse their hands in water, has many causes. It is worsened by washing dishes, clothes, and babies because repeated exposure to even mild detergents and water or prolonged sweating under rubber gloves may irritate dermatitic skin or may cause a marginal contact dermatitis.

Pompholyx is a chronic condition characterized by deep-seated pruritic vesicles on the palms, sides of the fingers, and soles. Scaling, redness, and oozing often follow vesiculation. The condition is also known as **dyshidrosis**--a misnomer because sweating may be decreased, normal, or excessive. Although most cases are idiopathic, a cause (eg, fungal infection, id reaction, allergic reaction, atopy) should always be sought.

Psoriasis localized to the hands may present on the dorsum as typical thick, silvery, scaling papules or plaques, but palmar lesions may be atypical. Although pitted grooves in the nails often indicate psoriasis,

they can occur with any dermatitis.

Recalcitrant pustular eruptions of the palms and soles are characteristically deep-seated sterile pustules of unknown cause that resist treatment. They may be associated with psoriasis elsewhere (Barber's or pustular psoriasis).

Fungal infection is common on the feet but uncommon on the hands. Patients with a hand dermatitis should be examined for a fungal infection of the feet (see Dermatophyte Infections in Ch. 113).

Diagnosis and Treatment

Diagnosis is made by microscopic examination of a scraping in 20% potassium hydroxide solution. Treatment should be directed at removing the cause whenever possible. The general principles of treatment listed in Table 111-2 are useful if no specific cause is found.

Lichen Simplex Chronicus

(Localized Scratch Dermatitis; Neurodermatitis)

A chronic, superficial, pruritic inflammation of the skin, characterized by dry, scaling, well-demarcated, hyperpigmented, lichenified plaques (thickened skin with accentuated markings) of oval, irregular, or angular shape.

Etiology, Symptoms, and Signs

The disease is a vicious cycle of itch begetting scratch begetting itch. Allergy appears to play no part. It affects more women than men, with onset usually between ages 20 and 50. It is rare in blacks but common in Asians and American Indians.

From prior irritation or without apparent reason, an area of skin begins to itch recurrently. The most frequently involved sites are the occiput, arms, and legs. Vigorous scratching gives only transient relief or exacerbates itch. Stress and tension increase the pruritus, and scratching may become an unconscious habit. The usual course is chronic.

Diagnosis

Diagnosis can usually be made by inspection--a fully developed plaque has an outer zone of brownish discrete papules and a central zone of confluent papules covered with scales. Underlying causes should be excluded because generalized pruritus without apparent skin lesions may occur in patients with various systemic disorders (see Pruritus in Ch. 109).

Treatment

The patient should be taught that scratching and rubbing produce the skin changes. The cycle of itching and scratching must be broken. The pruritus may be controlled most effectively with topical corticosteroids; a

cream may be rubbed in, or surgical tape impregnated with flurandrenolide (applied in the morning and replaced in the evening) may be preferred because it also prevents scratching. Small areas may be locally infiltrated with a long-acting corticosteroid such as triamcinolone acetonide 2.5 mg/mL (diluted with saline), 0.3 mL/cm^2 of lesion; this can be repeated q 3 to 4 wk. Oral H_1-blocking antihistamines or doxepin 10 mg at bedtime, increased to 25 to 50 mg/day if tolerated, may be useful.

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